Rapid Report

Reaction of nitric oxide and its derivatives with sulfites: a possible role in sulfite toxicity

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Abstract

The reaction between sulfites and nitric oxide or proposed carriers of nitric oxide (nitrosylated bovine serum albumin and S-nitrosoglutathione) was investigated as a potential source of the adverse effects of sulfites on biological systems. Rapid reaction occurred between sulfites and all of these reagents. Also, the ability of nitric oxide and these carriers of nitric oxide to inhibit platelet aggregation was reversed by low concentrations of sulfites. Counteraction of nitric oxide's ability to function in cell signaling processes may be a major cause of sulfite toxicity.

Keywords: Nitric oxide; Nitric oxide derivative; Sulfite toxicity; Platelet aggregation; Signaling; Respiratory disease

Sulfites (widely defined as the sulfiting agents, sulfur dioxide gas, metabisulfite, bisulfite and sulfite) are often encountered in the environment. Exposure to sulfites may result from inhaling sulfur dioxide which may be converted to the other sulfites upon contact with fluids in the air passages [1,2] or as the result of the ingestion of foods and drugs treated with some type of sulfiting agent [1,3–5]. Airborne concentrations of sulfur dioxide as high as 0.23 ppm have recently been reported and have correlated with transient increases in respiratory and heart disease [6,7]. Sulfiting agents are used as preservatives in foods, alcoholic beverages and drugs at concentrations as high as 4000 ppm [1] and studies have shown that metabisulfite, bisulfite and sulfur dioxide all can cause bronchoconstriction [3–5]. Persons with no history of asthma can experience respiratory distress in the presence of high concentrations of sulfites while asthmatics are particularly sensitive to sulfites [3–6,8].

The mechanism of action of sulfites in precipitating asthmatic episodes has not been elucidated. However, treatment with drugs such as atropine can alleviate the effects of sulfites [5,8]. Since these drugs act on the parasympathetic pathway, it was suggested that sulfites may affect some mediator within this pathway [3,5,8].

Nitric oxide (NO) functions as a neurotransmitter, it inhibits platelet aggregation and it regulates smooth muscle tone, thereby mediating blood pressure [9–13]. It has a short half-life in biological fluids, so carriers of nitric oxide such as S-nitrosoglutathione and S-nitrosylated serum albumins have been suggested [16,17,21–23]. It has previously been shown that the stimulation of muscarinic receptors can initiate the production of nitric oxide [13–18], followed by the upregulation of its target enzyme, guanylate cyclase [10,19,20]. Thus nitric oxide can be considered to be a messenger within the parasympathetic pathway. The results of this study suggest that the reaction of nitric oxide and its carriers with sulfites may be a major source of sulfites' biological toxicity.

In the absence of oxygen, the partial pressure of nitric oxide in solution can be measured directly using a Clark-type oxygen probe [26]. In Fig. 1, aliquots of sulfites solution (1 μL, 0.5 M sulfites) were added to a nitric oxide solution at the times indicated by the arrows. The rate of change appeared similar to the time of mixing and the response time of the instrument. Addition of the same quantity of aerated water (1 μL) produced a barely detectable signal change. In order to test whether the probe was functioning properly, sulfites were added to bring the probe reading to zero, followed by addition of known amounts of either nitric oxide solution or oxygenated buffer. These additions produced the expected response. Fig. 1 clearly demonstrated a rapid reaction of nitric oxide

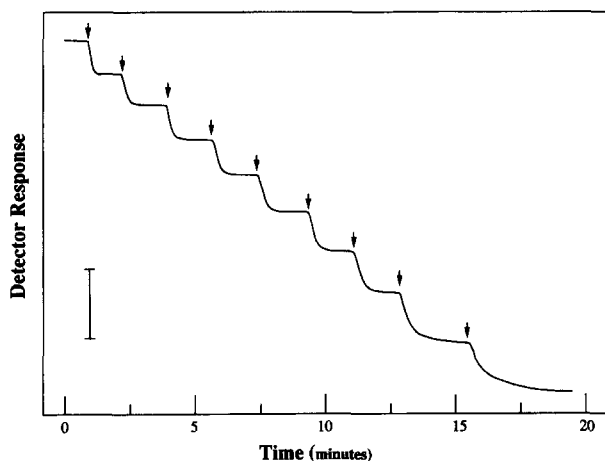* Corresponding author. E-mail: gary-N@molbio.cbs.umn.edu. Fax: +1 612 6255780.

Fig. 1. The reaction of nitric oxide with sulfites. Nitric oxide solution was made by bubbling nitric oxide gas (98.5%, Aldrich Chem. Co.) for 15 min into phosphate buffer (50 mM phosphate, 0.1 M NaCl (pH 7.0)) that had been purged of oxygen with N_2 gas for 1 h. The nitric oxide partial pressure of this solution (10 mL) was monitored with a Biological Oxygen Monitor and probe (Yellow Springs Instrument Co.) and is shown on the vertical axis. Nitric oxide gives 1/3 the response of oxygen in this system (manufacturer's estimate). A solution of sulfites (1 μL of 0.5 M sulfites made from sodium metabisulfite) was added at each arrow using a gas tight syringe fitted with a 22-gauge Teflon tube. Exposure to air was minimized and the solution was stirred continuously at room temperature. The vertical bar at the lower left indicates the response of the moniter to 53 μM oxygen, determined by a standard procedure [32].

with sulfites. The dinitrososulfite ion, a 2:1 divalent anion complex of nitric oxide and sulfite, is known to form when nitric oxide gas is passed through basic solutions of sulfites [24]. Though not rigorously determined, the stoichiometry of the reaction between nitric oxide and sulfites as determined from data such as that in Fig. 1, ranged from 1.34:1 to 2:1 for various additions and experiments.

Nitric oxide has a short half-life in extracellular media and may not function as a paracrine-type messenger itself, but in the form of a complex with carrier molecules [16,17,23]. Fig. 2 shows the results of reaction of sulfites with S-nitrosylated bovine serum albumin, one of the proposed carriers of nitric oxide [21,23]. The S-nitrosyl derivative can be measured spectrophotometrically at 335 nm [21–23]. Sulfites clearly reacted with the nitrosylated thiols and bleached the chromophore (Fig. 2).

The rate and extent of bleaching of the chromophore at 335 nm was dependent on the amount of sulfites added but not in direct ratio. That is, doubling of sulfites did not produce twice as much bleaching. This suggested the possibility of a heterogeneous population of nitrosylated groups, some of which were readily susceptible to sulfites while others were not. Sulfites can also react with disulfide bonds to produce free sulfhydryls that may undergo S-nitrosothiol–thiol exchange [22,27]. Bovine serum albumin has 17 disulfide bonds and one free sulfhydryl group [28,29], so that a heterogeneous population is possible. While the question of heterogeneity was not addressed

further, subsequent results showed that bleaching of absorbance at 335 nm was correlated with the loss of impact of nitrosylated bovine serum albumin on platelets. Studies, similar to those in Fig. 2, demonstrated reaction of sulfites with S-nitrosoglutathione, another proposed carrier of nitric oxide (not shown).

Since sulfites indeed react with nitric oxide and with carriers of nitric oxide, then the presence of sulfites should reverse the ability of nitric oxide to function in biological processes. An example of a nitric oxide effect is the inhibition of platelet aggregation [10,23,25]. In Fig. 3, a solution of nitric oxide effectively inhibited the onset of all stages of aggregation. Sulfites added prior to nitric oxide lessened its effect on platelet aggregation in a concentration-dependent manner. The sequence of addition (nitric oxide, thrombin or sulfites; data not shown) had only minor effects on this pattern of behavior. In Fig. 4, platelet aggregation was inhibited by S-nitrosoglutathione. Again, sulfites reversed this effect. Prior incubation of S-nitrosoglutathione with sulfites for 15 min nearly abolished the efficacy of S-nitrosoglutathione (tracing 4, Fig. 4). Results similar to those presented in Fig. 4 were also obtained when the S-nitrosylated bovine serum albumin was used in place of S-nitrosoglutathione (result not shown). The concentrations of sulfites used in these experiments had no detectable effect on platelet aggregation (Fig. 4, tracing 5). At high concentrations ($\geq$ 1.5 mM), sulfites became in-
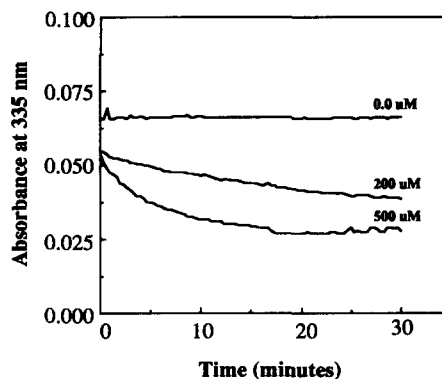

Fig. 2. Reaction of S-nitrosylated bovine serum albumin with sulfites. S-nitrosyl bovine serum albumin was produced following a published procedure [23]. The bovine serum albumin (10 mg/mL in 10 mM Hepes/0.1 M NaCl (pH 7.4), approx. 20 mL) was dialyzed for 1 h against Hepes buffer (100 mL) that was constantly purged with N_2 gas. Nitric oxide gas was then passed through the dialysis buffer for 15 min. Finally, the protein solution was dialyzed against 2 L Hepes buffer for 18 h. The absorption spectrum of the derivatized serum albumin showed a peak at 335 nm ($A = 0.066$), characteristic of S-nitrosylated bovine serum albumin. The protein concentration and the published extinction coefficient at 335 nm [22] gave a stoichiometry of 0.5 mol of S-nitrosylated derivative per mol of serum albumin. The change in absorbance at 335 nm after addition of sulfites solution was followed at room temperature using a Beckman DU 70 spectrophotometer. The uppermost tracing represents the absorbance with no sulfites added, the second and third tracings show the result after addition of 200 and 500 μM sulfites, respectively.
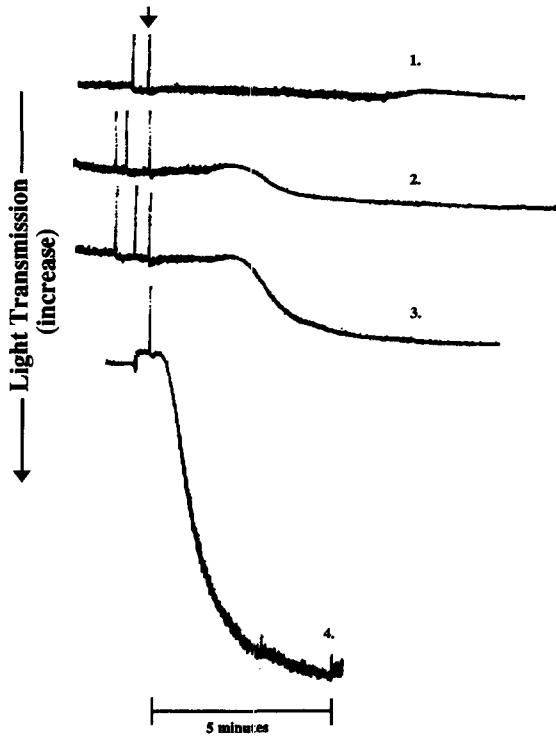
Fig. 3. Effects of sulfites on nitric oxide inhibition of platelet aggregation. Platelet concentrate (Memorial Blood Center of Minneapolis) was used on the 6th or 7th day after being drawn. The platelets were constantly agitated during storage and were washed by standard procedures [31] just before use. Various preparations used in this study contained 500000 ± 100000 platelets per μL and were used undiluted. Platelet aggregation was monitored using a Chrono-Log 330 platelet aggregometer at 37° C. Platelet aggregation was initiated by addition of 40 mU of bovine thrombin (2000 U/mg, prepared as in [30]) at the arrow. Sulfites and/or nitric oxide were added less than 1 min prior to thrombin. Tracing 1 shows the results when 4 μM nitric oxide was added prior to thrombin. Tracing 4 shows the control experiment without nitric oxide. Tracings 2 and 3 show the results for reactions that contained nitric oxide (4 μM) plus 30 μM or 100 μM sulfites (sodium metabisulfite), respectively. The tracings have been offset for clarity.

surprising. Although not established in this study, it is possible that the direct reaction of nitric oxide and sulfites proceeds to a known complex [24]. This study found that sulfites also react with S-nitroso compounds. Again, while reaction mechanisms and products can readily be postulated, further work will be needed to establish the exact chemistry and products formed from these reactions in dilute buffered solutions.

Nitric oxide is used not only as a messenger, it is also used as a bactericide, and excessive production of nitric oxide is toxic. Septic shock, a situation were the smooth muscle surrounding blood vessels relax excessively, is a potential result of an overproduction of nitric oxide [10]. If sulfites indeed react with nitric oxide in biological systems, they might function to reverse such effects.
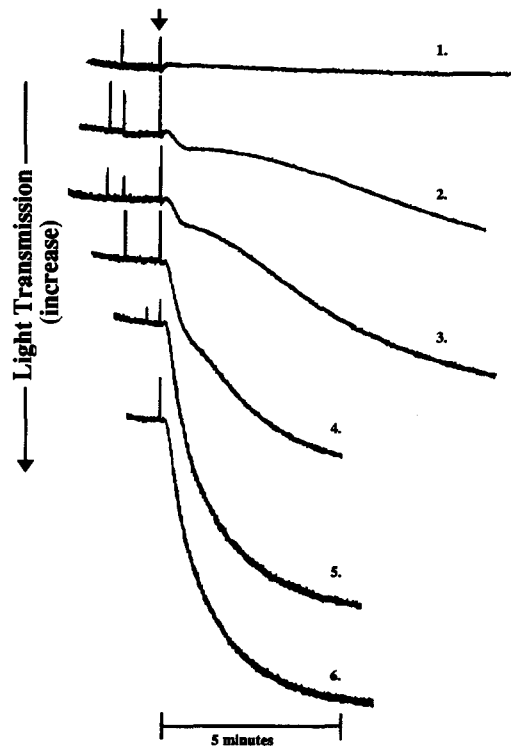
hibitors of platelet aggregation (not shown), so that their opposite impact at low concentration and in the presence of nitric oxide (Figs. 3 and 4) appeared to stem from reaction with nitric oxide or its derivatives.

Very low concentrations of nitric oxide carriers are highly effective at mediating nitric oxide's physiological effects [21,23] and 600 nM S-nitrosoglutathione (Fig. 4) represented an excess that inhibited platelet aggregation completely. That micromolar concentrations of sulfites partially reversed the effects of excess reagent suggested that much lower concentrations of sulfites would influence the affects of a minimum dose of nitric oxide reagent. Furthermore, it is possible that other nitric oxide response systems are even more sensitive to sulfites. Thus, the nitric oxide signaling pathway may be exquisitely sensitive to sulfites.

Both sulfites and nitric oxide are highly reactive inorganic materials so that rapid mutual interaction is not

Fig. 4. Effects of sulfites on S-nitrosoglutathione inhibition of platelet aggregation. Platelets were prepared as outlined in Fig. 3. Platelet aggregation was initiated in each case by the addition of 20 mU thrombin at the arrow. S-nitrosoglutathione (600 nM, from Molecular Probes), added before thrombin, gave the results shown in the tracing 1. Tracing 6 shows the comparable experiment but without S-nitrosoglutathione. Tracings 2 and 3 show the results when S-nitrosoglutathione (600 nM) plus 50 or 100 μM sulfites, respectively, were added before the thrombin. Tracing 4 shows the result of the addition of S-nitrosoglutathione (600 nM) that had been pre-incubated for 15 min in a solution that contained 200 μM sulfites and 100 μM S-nitrosoglutathione. In this experiment, the concentration of sulfites in the final platelet solution was 1.2 μM, inadequate to have a detectable effect on 600 nM S-nitrosoglutathione if it were added directly to the platelet solution. Tracing 5 shows the result when 100 μM bisulfite and thrombin were the only additives. The tracings have been offset for clarity.

Overall, this study has shown that sulfites are capable of reacting not only with nitric oxide but also with several of its derivatives. This reaction affected nitric oxide's ability to function as a physiological messenger. While the speed of reaction and sensitivity of nitric oxide to sulfites make it seem likely that this is a major source of sulfite toxicity, further studies will be needed to determine the quantitative contributions of this mechanism to the adverse reactions caused by sulfiting agents.

Acknowledgements

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