JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Effects of the Food Additive Sulfite on Nitrite-Dependent Nitric Oxide Production under Conditions Simulating the Mixture of Saliva and Gastric Juice

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ABSTRACT: The food additive sulfite is mixed with saliva, which contains nitrite, in the oral cavity, and the mixture is mixed with gastric juice in the stomach. In the stomach, salivary nitrite can be transformed to nitric oxide (NO). In this study, the effects of sulfite on nitrite-dependent NO production were investigated using acidified saliva (pH 2.6) and acidic buffer solutions (pH 2.0). Sulfite enhanced NO production in acidified saliva and acidic buffer solutions, and the enhancement increased with the increase in sulfite concentration from 0 to 0.1 mM, whereas suppressed NO production and the suppression increased as the concentration was increased over 0.2 mM. The enhancement was due to the increase in reaction rate between nitrous acid and nitrososulfonate ($ONSO_3^-$) that was formed by the reaction of nitrous acid with hydrogen sulfite, and the suppression was due to the increase in hydrogen sulfite-dependent consumption rate of $ONSO_3^-$. A salivary component SCN^- (1 mM) enhanced and suppressed NO production induced by 1 mM nitrite when sulfite concentrations were lower and higher than 1 mM, respectively. $ONSO_3^-$ formed from hydrogen sulfite and nitrosyl thiocyanate (ONSCN), which was produced by the reaction of nitrous acid with SCN^-, seemed to contribute to the enhancement and suppression. NO production induced by nitrite/ascorbic acid systems was suppressed by sulfite, and the suppressive effects were decreased by SCN^-, whereas sulfite-induced suppression of NO production in nitrite/rutin systems was increased by SCN^-. During reactions of nitrite with sulfite in the presence and absence of SCN^-, oxygen was taken up. The oxygen uptake is discussed to be due to autoxidation of NO and radical chain reactions initiated by hydrogen sulfite radicals. The results of the present study suggest that sulfite can enhance and suppress nitrite-dependent NO production. It is discussed that radicals including hydrogen sulfite radicals can be formed through the reactions of nitrite and sulfite in the stomach.

KEYWORDS: *ascorbic acid, nitrate formation, oxygen uptake, rutin, SCN^-, sulfate formation*

INTRODUCTION

Sodium sulfite is added to various foods and beverages as an antioxidant and a preservative, and sulfite concentrations range from 0.03 to 1.5 g/kg. Because the pK_a values of sulfurous acid (H_2SO_3) are 1.81 and 6.97,[1] H_2SO_3 and hydrogen sulfite ion (HSO_3^-) are present in the stomach, the pH of which is around 2[2] after the ingestion of sulfite-containing foods and beverages. HSO_3^- can autoxidize, and the autoxidation is enhanced by $HO_2^\bullet$ (pK_a = 4.9) and transition metal ions.[3] During the oxidation of HSO_3^-, $HSO_3^\bullet$ (pK_a = 7.2),[4] which reacts with O_2 to generate $HSO_5^\bullet$, is formed.[3] $HSO_5^\bullet$ oxidizes HSO_3^- to $HSO_3^\bullet$ via $HSO_4^\bullet$ to continue the radical chain reactions.

Nitrate, which is mainly derived from plant foods, is present in human saliva, and salivary nitrate is reduced to nitrite by nitrate-reducing bacteria in the oral cavity.[5−8] The concentration of nitrite in mixed whole saliva is 0.05−1 mM.[5] When foods and beverages that contain sulfite are ingested, sulfite is mixed with nitrite in the oral cavity; subsequently, the mixture of sulfite and nitrite is mixed with gastric juice in the stomach. If pH in the stomach ranges from 2 to 4 after the ingestion of foods or beverages, 20−95% of nitrite is present as HNO_2 (pK_a = 3.3)[4] and 60−100% of sulfite is present as HSO_3^-.[1] Because HSO_3^- reacts with HNO_2 to produce nitrososulfonate ion ($ONSO_3^-$)[9−11] (Figure 1), such reaction between sulfite and nitrite is possible in the stomach. Three pathways have been proposed for the transformation of $ONSO_3^-$. The first pathway is the reaction of $ONSO_3^-$ with HSO_3^- to produce the

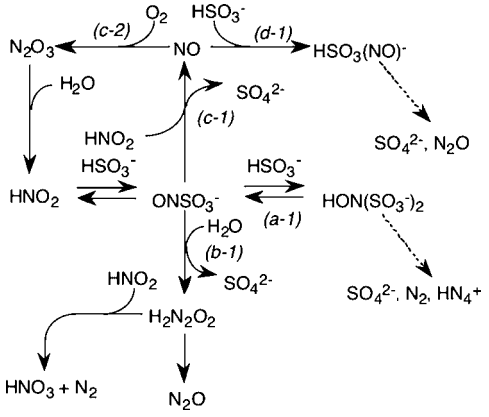

Figure 1. Possible reactions of sulfite and nitrite under acidic conditions. The scheme was prepared with reference to work cited in the text.

Received: September 18, 2011
Revised: December 28, 2011
Accepted: January 4, 2012
Published: January 4, 2012

hydroxylamine disulfonate [$HON(SO_3^-)_2$] (reaction *a-1*), which can be transformed to SO_4^{2-}, N_2, and NH_4^+ (shown as dashed lines). The second pathway is hydrolysis of $ONSO_3^-$ to produce SO_4^{2-} and hyponitrous acid ($H_2N_2O_2$) ($pK_a = 7.21$)[4] that is transformed to HNO_3, N_2, and N_2O (reaction *b-1*). The third pathway is the reaction of $ONSO_3^-$ with HNO_2 to produce SO_4^{2-} and nitric oxide (NO) (reaction *c-1*). NO produced is oxidized to N_2O_3 by reaction *c-2*[12−15] or transformed to hydroxy(nitroso)sulfamate [$HSO_3(NO)^-$] by reacting with HSO_3^- (reaction *d-1*).[1,16]

As shown in Figure 1, reactions of nitrite with sulfite under acidic conditions have been studied extensively in abiotic systems, but the effects of SCN^- on the reaction between sulfite and nitrite have not been studied to the authors' knowledge. After ingestion of sulfite-containing foods or beverages, sulfite is mixed with salivary nitrite and SCN^-[17] in the oral cavity and the pH of the mixture is decreased in the stomach. Therefore, this paper initially deals with sulfite-induced NO production and O_2 uptake in acidified saliva that simulated the mixture of saliva and gastric juice and then deals with the effects of SCN^- on sulfite-induced NO production and O_2 uptake in acidic buffer solutions to elucidate the mechanism of NO production and O_2 uptake in acidified saliva. Furthermore, this paper deals with the effects of sulfite on NO production in nitrite/ascorbic acid and nitrite/polyphenol systems under acidic conditions, because ascorbic acid is a component of gastric juice and polyphenols are contained in various foods and beverages to which sulfite is added. By considering the results obtained in this study, the effects of sulfite on nitrite-dependent NO production in the stomach are discussed.

MATERIALS AND METHODS

Reagents. *N*-(Dithiocarboxy)sarcosine sodium salt (DTCS) and an NO-generating reagent, 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC 7) (purity > 90%), were obtained from Dojin (Kumamoto, Japan). Polyphenols (caffeic acid, chlorogenic acid, rutin, quercetin) and Griess−Romijn reagent for the determination of nitrite were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of Saliva. Mixed whole saliva (approximately 10 mL) was collected from volunteers by chewing parafilm between 9 and 10 o'clock in the morning after informed consent had been obtained. The collected saliva was passed through two layers of nylon filter nets [380 mesh (32 μm) net, Sansho, Tokyo, Japan] to remove epithelial cells. The filtrate was used as saliva. Nitrite and SCN^- in the filtrate were quantified using Griess−Romijn reagent and $FeCl_3$, respectively, as previously reported.[18]

O_2 Uptake. Changes in O_2 concentration were recorded using a Clark-type electrode (Rank Brothers, Cambridge, U.K.) at 30 °C. The polarizing voltage was −0.6 V. The reaction mixture contained 1.33 mL of saliva filtrate and 0.66 mL of 50 mM KCl−HCl (pH 1.3). The pH of the mixture was 2.6−2.7, and the concentrations of nitrite and SCN^- were 0.20−0.22 and 0.09−0.2 mM, respectively. The mixture was incubated for defined periods, following which sodium sulfite was added. O_2 uptake in buffer solutions was studied using reaction mixtures (2 mL) containing various concentrations of sodium sulfite and sodium nitrite in 50 mM KCl−HCl (pH 2.0).

Measurements of NO Production. It has been reported that NO is trapped by $Fe(DTCS)_3$ around neutral pH and that the yield of NO-$Fe(DTCS)_2$ is 40% in a sodium phosphate saline solution.[19] Then, production of NO was measured using an electron spin resonance (ESR) spectrometer (JE1XG, JEOL; Tokyo, Japan) at 25 °C with a quartz flat cell (0.05 mL). ESR spectra were recorded under the following conditions:[20,21] microwave power, 10 mW; scanning speed, 5 mT/min; line width, 0.5 mT; and amplification; 1000- or 2000-fold depending on ESR signal intensity. $Fe(DTCS)_3$ solution was prepared by adding 0.03 mL of 100 mM $FeCl_3$ to 1 mL of 10 mM DTCS, which was dissolved in 0.1 M sodium phosphate (pH 7.6).

NO production in acidified saliva was studied in the reaction mixture containing 0.166 mL of saliva and 0.084 mL of 50 mM KCl−HCl (pH 1.3). The pH and the concentrations of nitrite and SCN^- in the mixture were the same as those of acidified saliva used to study O_2 uptake. Acidified saliva was incubated for 1 min after the addition of sodium sulfite, and then 0.25 mL of $Fe(DTCS)_3$ was added. Immediately after the addition of $Fe(DTCS)_3$, an aliquot of acidified saliva was withdrawn into the quartz flat cell to record ESR spectra. Because $Fe(DTCS)_3$ could not react with NO at pH 2, $Fe(DTCS)_3$ was added after the incubation of acidified saliva for 1 min. The pH after the addition of $Fe(DTCS)_3$ was approximately 7.3.

NO production was also studied in 50 mM KCl−HCl (pH 2.0) (0.25 mL) that contained nitrite and sulfite. The reaction mixture was incubated for 1 min, after which 0.25 mL of $Fe(DTCS)_3$ was added. The pH after the addition of $Fe(DTCS)_3$ was approximately 7.2.

When NO production was measured under anaerobic conditions using $Fe(DTCS)_3$, O_2 in 0.25 mL of 50 mM KCl−HCl (pH 2.0) that contained sulfite was removed by bubbling argon gas through the reaction mixture for 1−2 min. The establishment of anaerobic conditions was ascertained by monitoring the decrease in O_2 concentration using an O_2 electrode. The anaerobic mixture was incubated for 1 min without bubbling after the addition of nitrite, and then 0.25 mL of $Fe(DTCS)_3$ was added to generate NO-$Fe(DTCS)_2$. Because rate constants of the reactions between NO and O_2 and between NO and Fe-$(DTCS)_3$ are $(2-6) \times 10^6$ M^{-2} s^{-1}[13,15] and 4.8×10^8 M^{-1} s^{-1},[22] respectively, almost all NO present in the reaction mixture can be trapped by added Fe-$(DTCS)_3$. Evolution of NO to the atmosphere would be negligible because solubility of NO in water is 1.9 mM at 25 °C.

NO production was also measured in 50 mM KCl−HCl (pH 2.0) at 30 °C using a Clark-type electrode. The polarizing voltage was −0.7 V. After O_2 had been excluded from buffer solutions by bubbling argon gas through the solution, NO production was recorded by adding nitrite, sulfite, and SCN^-. NO production was also studied in nitrite/ascorbic acid and nitrite/polyphenol systems. NOC 7 (0.1 mM) was used to calibrate NO concentration.

Spectrophotometric Measurements. Spectrophotometric measurements were performed using a model 557 (Hitachi, Tokyo, Japan) or a model UV-260 (Shimadzu, Kyoto, Japan) spectrophotometer. The path length of the measuring beam was 4 mm.

The solubility of barium sulfate is very low. Therefore, the formation of SO_4^{2-} was estimated by monitoring the apparent absorbance increase at 600 nm in the presence of barium ion. The reaction mixture (1 mL) contained 0.25 mM sodium nitrite and 2 mM barium nitrate in 50 mM KCl−HCl (pH 2.0). Reactions were initiated by adding sodium sulfite under aerobic and anaerobic conditions. Anaerobic conditions were established by bubbling argon gas through the reaction mixture in a cuvette for 1−2 min.

Changes in absorption spectra of sulfite and nitrite were recorded in the reaction mixture (1 mL) containing 1 mM sodium sulfite or 1 mM sodium nitrite in 50 mM KCl−HCl (pH 2.0). Recordings were started after the addition of 0.4 mM sodium nitrite or 1 mM sodium sulfite to the former and the latter reaction mixtures, respectively.

Because effects of sulfite on NO production in nitrite/ascorbic acid and nitrite/polyphenol systems were studied under anaerobic conditions, nitrite-induced changes in absorption spectra of ascorbic acid and polyphenols were also studied under anaerobic conditions. Absorbance changes of ascorbic acid were studied in the reaction mixture (1 mL) containing 0.1 mM ascorbic acid and 0.2 mM nitrite in 50 mM KCl−HCl (pH 2.0). Sulfite (1 mM) and NaSCN (1 mM) were added as required. Nitrite-induced oxidation of rutin was also studied in the reaction mixture (0.5 mL) containing 0.5 mM rutin and 0.5 mM nitrite in 50 mM KCl−HCl (pH 2.0). Because the absorbance of 0.5 mM rutin at 350 nm was 1.5 (path length of measuring beam, 2 mm) and nitrite-induced absorbance changes were slow, the oxidation of rutin was studied by recording difference spectra before and after the addition of nitrite. Sulfite and NaSCN were added as required.

The formation of nitrosyl thiocyanate (ONSCN) was studied in the reaction mixture that contained 10 mM $NaNO_2$ and 10 mM NaSCN in 0.2 M KCl–HCl (pH 1.3).[21,23] Na_2SO_3 (1 M) was prepared using 1 M HCl to prevent the increase in pH when sulfite solution was added to the above reaction mixture. The path length of the measuring beam was 10 mm.

Detection of Nitrate. Nitrate formed in sulfite/nitrite systems was separated using a Shim-pack CLC-C_8 column (6 mm i.d. × 15 cm) (Shimadzu) and detected at 210 nm using a spectrophotometric detector with a photodiode array (SPD-M10A, Shimadzu). The mobile phase was a mixture of methanol and 25 mM KH_2PO_4/H_3PO_4 (pH 3.0) (1:4, v/v), and the flow rate was 1 mL/min. The reaction mixture (1 mL) contained 0.25 mM sodium nitrite with and without NaSCN in 50 mM KCl–HCl (pH 2.0). Reactions were initiated by adding sulfite. After incubation for 1 or 2 min, 10 μL of the reaction mixture was applied to the HPLC column. When required, anaerobic conditions were established by bubbling argon gas as described above. The formation of nitrate and the consumption of nitrite were estimated from the areas under peaks.

Data Presentation. Each experiment was repeated at least twice. The averages, means with SDs, or typical data of time courses and changes in absorption spectra were presented.

RESULTS

Effects of Sulfite on NO Production and O_2 Uptake in Acidified Saliva. Addition of $Fe(DTCS)_3$ to acidified saliva, which had been incubated for 1 min in the presence and absence of sulfite, resulted in the development of a stable signal corresponding to NO-$Fe(DTCS)_2$ (g = 2.04)[19] (Figure 2, top, inset), indicating NO production in the acidified saliva as previously reported.[20,21] The formation of NO-$Fe(DTCS)_2$ increased when the sulfite concentration was increased from 0 to 0.1 mM, attaining a maximal value, and subsequently decreased (Figure 2, top).

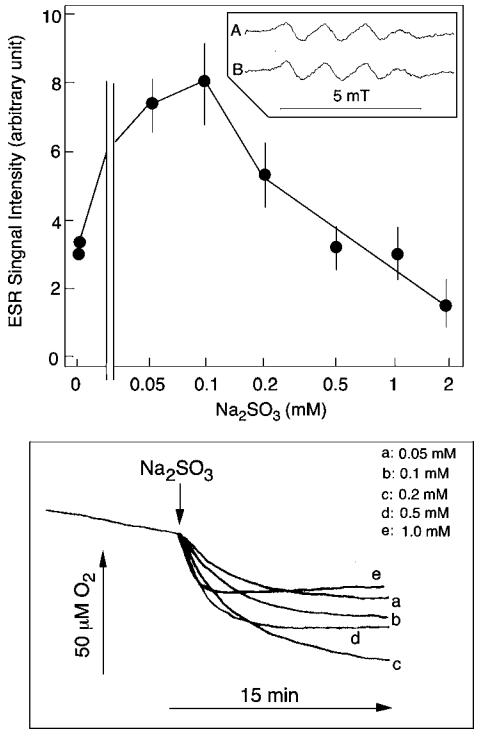

Figure 2. Sulfite-induced formation of NO-$Fe(DTCS)_2$ and O_2 uptake in acidified saliva. (Top) Formation of NO-$Fe(DTCS)_2$ as a function of sulfite concentration. Each data point is the mean with SD (n = 3). (Inset) Typical ESR spectra of NO-$Fe(DTCS)_2$ (amplification, 2000-fold) (A) immediately after the addition of $Fe(DTCS)_3$ to a sample and (B) 2.5 min after the addition of $Fe(DTCS)_3$. (Bottom) Time courses of sulfite-induced O_2 uptake. Downward arrow indicates the addition of various concentrations of Na_2SO_3.

Slow O_2 uptake was observed in acidified saliva (Figure 2, bottom). Sulfite enhanced the O_2 uptake, and the rate observed after the addition of sulfite increased with the increase in sulfite concentration, attaining a constant value. The amount of O_2 uptake increased when sulfite concentration was increased from 0 to 0.2 mM (traces a–c) and then decreased when increased from 0.2 to 1 mM (traces c–e). Because NO was produced in acidified saliva, part of the O_2 uptake could be attributed to autoxidation of NO (reaction *c-2*). NO-dependent O_2 uptake has been observed in acidified saliva in the presence of ascorbic acid, chlorogenic acid, and quercetin.[20,21] The sulfite-dependent increase and decrease in NO production and O_2 uptake in acidified saliva prompted us to study NO production and O_2 uptake by sulfite/nitrite systems in acidic buffer solutions.

NO Production and O_2 Uptake by Nitrite/Sulfite Systems. No ESR signals of NO-$Fe(DTCS)_2$ were detected upon addition of $Fe(DTCS)_3$ to an acidic solution of sulfite or an acidic mixture of sulfite and nitrate. A stable and small signal corresponding to NO-$Fe(DTCS)_2$ was observed upon the addition of $Fe(DTCS)_3$ to an acidic nitrite solution, and the signal intensity increased and decreased as a function of sulfite concentration (Figure 3, top). The formation of NO-$Fe(DTCS)_2$ by sulfite/nitrite systems was greater under anaerobic than aerobic conditions, and its formation under anaerobic conditions also increased and decreased as a function of sulfite concentration. The greater formation under anaerobic conditions was due to the absence of reaction *c-2*.

Figure 3 (middle) shows time courses of NO production measured electrochemically. Very slow NO production was observed when 1 mM nitrite was added. The slow NO production was enhanced, attaining a constant rate (approximately 60 μM/min), whereas the amount of its formation increased and then decreased as a function of sulfite concentration. O_2 uptake induced by 0.5 mM sulfite (A) and sulfite plus nitrate (B) was slow, and the rate was about 0.35 μM/min (Figure 3, bottom). It is known that autoxidation of sulfite and hydrogen sulfite is slow in the absence of initiators of radical reactions.[3] Nitrite-induced slow O_2 uptake was enhanced greatly by sulfite, and the enhancement became larger with the increase in sulfite concentration, attaining a constant rate (approximately 40 μM/min), whereas the amount of O_2 uptake increased to a maximal value and then decreased with the increase in sulfite concentration (C). The effects of sulfite on O_2 uptake were similar to those of sulfite on NO production, supporting the contribution of reaction *c-2* to O_2 uptake. Addition of 2 and 4 mM sodium sulfite increased the pH of 50 mM KCl–HCl (pH 2.0) by approximately 0.05 and 0.1, respectively, indicating that the suppression by sulfite was not due to the increase in pH of reaction mixtures. The results in Figures 2 and 3 indicate that sulfite can reduce nitrite to NO consuming oxygen in the mixture of saliva and gastric juice.

To elucidate the mechanism of O_2 uptake by sulfite/nitrite systems, NO production and O_2 uptake were studied using NOC 7 and a sulfite/nitrite system. NOC 7 (0.1 mM), which could produce 0.2 mM NO, consumed 45.3 ± 1.7 μM O_2 (mean with SD; n = 4) promptly after the addition to 50 mM KCl–HCl (pH 2.0). When NO production and O_2 uptake were measured in the mixture of 0.25 mM sulfite and 1 mM nitrite, the amounts were 28.6 ± 3.0 and 33.2 ± 2.8 μM (means with SDs; n = 4), respectively. The results indicate (i) that almost all

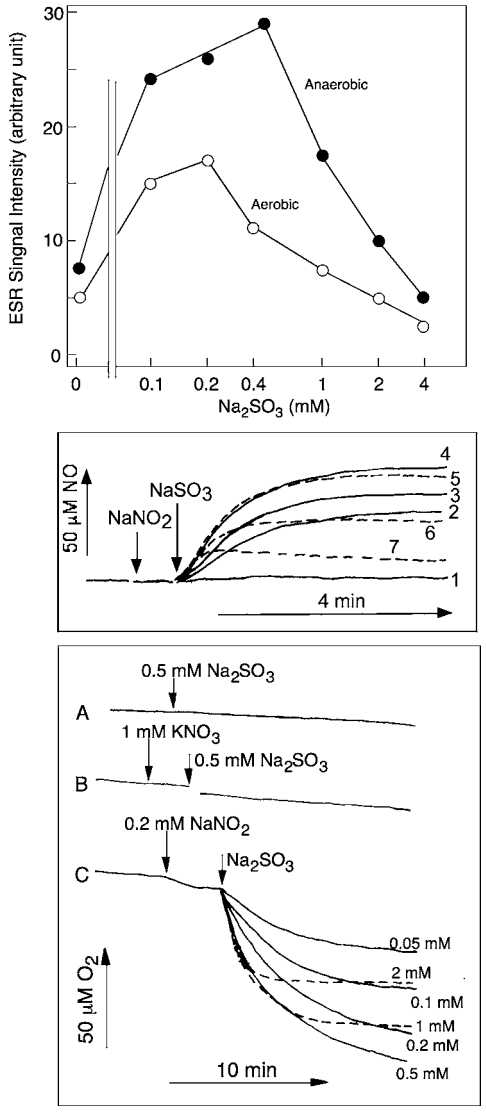

Figure 3. NO production and O_2 uptake by sulfite/nitrite systems. All reactions were run in 50 mM KCl–HCl (pH 2.0). (Top) NO-$Fe(DTCS)_2$ formation as a function of sulfite concentration in the presence of 1 mM $NaNO_2$: (○) aerobic; (●) anaerobic conditions. Each data point is the average of two experiments. (Middle) NO production measured electrochemically. One millimolar $NaNO_2$ and various concentrations of Na_2SO_3 were added where indicated by arrows: (1) without Na_2SO_3; (2) 0.1 mM; (3) 0.2 mM; (4) 0.5 mM; (5) 1 mM; (6) 2 mM; (7) 4 mM Na_2SO_3. (Bottom) O_2 uptake: (A) 0.5 mM Na_2SO_3; (B) 1 mM KNO_3 and 0.5 mM Na_2SO_3; (C) 0.2 mM $NaNO_2$ and various concentrations of Na_2SO_3. Arrows indicate the addition of components indicated in the figure.

of the NO produced by NOC 7 was autoxidized to N_2O_3 and (ii) that reactions other than autoxidation of NO contributed to the O_2 uptake in sulfite/nitrite systems.

Effects of SCN^- on NO Production and O_2 Uptake. Saliva contains 0.1–2 mM SCN^-.[17] Then, the effects of SCN^- on NO production in nitrite/sulfite systems were studied (Figure 4, left). The rate of NO production increased with the increase in SCN^- concentration, keeping the amount of NO produced nearly constant in the presence of 1 mM nitrite and 0.25 mM sulfite (top). The amount was estimated to be 30.1 ± 2.3 μM (mean ± SD; n = 4). SCN^- enhanced the initial rate but decreased the amount of NO produced when sulfite concentration was <1 mM and inhibited the initial rate as well as the amount of NO when sulfite concentration was >1 mM (bottom). The effects of SCN^- on O_2 uptake were also studied in a sulfite/nitrite system (Figure 4, right). The rate of O_2 uptake was enhanced by SCN^- (top and bottom), whereas the amount of O_2 uptake was decreased by SCN^- in the presence of higher concentrations of sulfite (>0.2 mM) (bottom). The results in Figure 4 indicate that SCN^- in saliva can enhance and suppress NO production in the stomach depending on the concentration of sulfite and support that not only autoxidation of NO but also other reactions contributed to O_2 uptake.

SCN^- reacts with nitrous acid as follows:[21,23]

$$HNO_2 + SCN^- + H^+ \rightleftarrows ONSCN + H_2O \quad (1)$$

Then, reactions of sulfite with ONSCN were studied to make clear the mechanism of SCN^--dependent enhancement of NO production (Figure 5). The absorbance of ONSCN around 460 nm decreased rapidly by sulfite, and the extent of the absorbance decrease was dependent on sulfite concentration. This result indicates that ONSCN formed by reaction 1 can rapidly react with hydrogen sulfite and/or sulfurous acid in the stomach.

Consumption of Sulfite and Nitrite by Sulfite/Nitrite Systems. During reactions of sulfite with nitrite, the concentrations of these components should be decreased. The absorbance of sulfite around 275 nm, at which nitrite had no absorption bands, was decreased upon the addition of nitrite, consuming about 0.6 mM sulfite by 0.4 mM sodium nitrite (Figure 6A-1). The absorbance of nitrite around 370 nm, where sulfite had no absorption bands, was decreased by sulfite, consuming about 0.62 mM nitrite by 1 mM sodium sulfite (B-1). The stoichiometries (consumption of 1 mol of nitrite by about 1.5 mol of sulfite) indicate the occurrence of reaction *a-1* in addition to reactions *b-1* and *c-1*.[10,11]

Rates of consumption of sulfite and nitrite were enhanced by SCN^- as expected from the results in Figures 4 and 5, but the extents of their consumption were not significantly affected by SCN^- (A-2 and B-2), suggesting that SCN^- did not affect so much the stoichiometry of the reaction between nitrite and sulfite under the conditions of Figure 6.

Formation of Sulfate by Nitrite/Sulfite Systems. Figure 7 shows the formation of sulfate by nitrite/sulfite systems. The addition of sulfite to an acidic mixture of nitrite and barium nitrate resulted in an absorbance increase at 600 nm with a lag (top). The absorbance increase was due to the formation of barium sulfate, and the lag was due to dissolution of barium sulfate into the buffer solution, the solubility of which in water was approximately 10 μM. SCN^- enhanced the absorbance increase in a concentration-dependent manner. The rate and amount of the absorbance increase became faster and larger, respectively, with the increase in sulfite concentration (middle). The bottom panel shows faster and greater formation of sulfate under anaerobic than under aerobic conditions in the presence and absence of SCN^-.

Nitrate Formation by Sulfite/Nitrite Systems. Figure 8 (left) shows HPLC of inorganic components used in this study. No significant peaks of 0.5 mM sulfite (trace B) and 0.5 mM sulfate (trace C) were detected. Peaks of nitrite (0.25 mM; trace D), nitrate (0.25 mM; trace E), and SCN^- (1 mM; trace F) were detected at retention times of 9, 3.1, and 3.7 min, respectively. The results indicate that nitrate formation is detectable under the conditions that sulfite and sulfate concentrations are not significantly higher than that of nitrate.

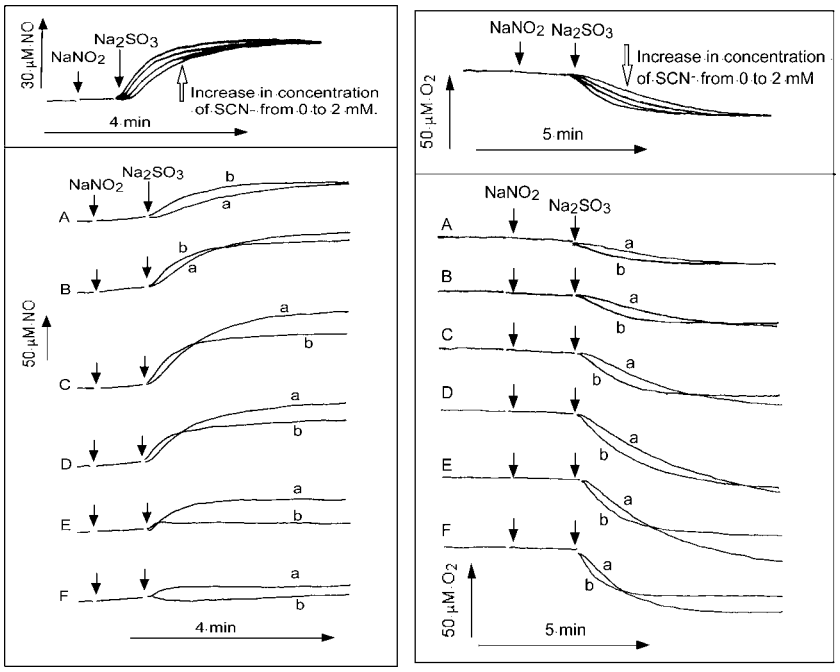

Figure 4. Effects of SCN^- on NO production and O_2 uptake in sulfite/nitrite systems. Reactions were run in 50 mM KCl−HCl (pH 2.0). (Left) NO production. (Top) SCN^- concentration dependency. Downward arrows indicate addition of 1 mM $NaNO_2$ and 0.25 mM Na_2SO_3. Prior to addition of these components 0, 0.2, 1, or 2 mM NaSCN was added. A white upward arrow indicates increase in concentration of NaSCN. (Bottom) Effects of Na_2SO_3 concentration (a) without NaSCN and (b) with 1 mM NaSCN. Downward arrows indicate addition of 1 mM $NaNO_2$ and various concentrations of Na_2SO_3: (A) 0.1; (B) 0.2; (C) 0.5; (D) 1; (E) 2; (F) 4 mM Na_2SO_3. (Right) O_2 uptake. (Top) SCN^- concentration dependency. The reaction mixture contained 0, 0.2, 1, or 2 mM NaSCN from the top to the bottom trace. Solid downward arrows indicate addition of 0.25 mM $NaNO_2$ and 0.1 mM Na_2SO_3. (Bottom) Effects of Na_2SO_3 concentration (a) without NaSCN and (b) with 1 mM NaSCN. Downward arrows indicate addition of 0.25 mM $NaNO_2$ and various concentrations of Na_2SO_3: (A) 0.05; (B) 0.1; (C) 0.2; (D) 0.5; (E) 1.0; (F) 2.0 mM Na_2SO_3.

No detectable decrease in nitrite peak was observed following incubation of 0.25 mM nitrite for 1 min in the presence and absence of SCN^- (Figure 8, middle, traces A and C; see also the caption). The incubation of 0.25 mM nitrite with 0.5 mM sulfite for 1 min resulted in the decrease in nitrite concentration by approximately 25% (trace B). SCN^- enhanced the decrease in nitrite concentration, and approximately 75% of nitrite was consumed during the incubation (trace D). Nitrate was generated in the presence and absence of SCN^- (traces B and D).

Concentrations of nitrite remaining under aerobic conditions were similar to those remaining under anaerobic conditions in sulfite/nitrite systems, which had been incubated for 2 min in the presence and absence of SCN^- (Figure 8, right; compare trace A with trace B and trace C with trace D). In contrast to this, nitrate was formed >2-fold under aerobic than anaerobic conditions. The larger formation of nitrate under aerobic conditions indicates that O_2 contributed to the oxidation of nitrite to nitrate.

Inhibition of NO Production by Sulfite in Nitrite/Ascorbic Acid Systems. Ascorbic acid (0.05−0.1 mM) is contained in gastric juice.[24] Figure 9 shows the effects of sulfite on NO production in nitrite/ascorbic acid systems. Nitrite induced slow NO production (top, trace 1), and the NO production was enhanced by 0.1 mM ascorbic acid (trace 2). The rate constant of the NO production was calculated from the initial rate by postulating that 1 mol of ascorbic acid reduced 1 mol of nitrous acid to produce NO and ascorbic acid radical. The value was $(25.0 \pm 6.4) \times 10^2$ M^{-1} min^{-1} (mean with SD, $n = 4$). In addition to NO production induced by 0.1 mM ascorbic acid, 46.7 ± 1.9 μM O_2 (mean with SD, $n = 3$) was taken up. Because ascorbic acid radical is transformed to ascorbic acid and dehydroascorbic acid, 0.1 mM ascorbic acid can reduce 0.2 mM nitrous acid to produce 0.2 mM NO. Therefore, the amount of O_2 taken up indicates that almost all of the NO produced was autoxidized to N_2O_3.

SCN^- enhanced NO production without affecting the amount of its production (trace 4), indicating rapid reaction of ascorbic acid with ONSCN produced by reaction 1.[25] The rate constant of reaction between ascorbic acid and ONSCN to produce NO, SCN^-, and dehydroascorbic acid has been reported to be 30×10^7 M^{-1} min^{-1}.[25] Sulfite suppressed NO production in nitrite/ascorbic acid systems (trace 3), and the suppression increased with the increase in sulfite concentration (Figure 9, bottom). SCN^- decreased the suppressive effects of sulfite (compare traces 4 and 5 in the top and open and solid circles in the bottom).

Ascorbic acid had an absorption peak at 243 nm in 50 mM KCl−HCl (pH 2.0). The initial rate of its oxidation in a nitrite/ascorbic acid system was hardly suppressed by 1 mM sulfite, but its oxidation was ceased when about 35% of ascorbic acid had been oxidized (compare panels A and B in Figure 10). During the inhibition of ascorbic acid oxidation, sulfite oxidation was observed as an absorbance decrease around 280 nm. The initial rate of oxidation of ascorbic acid in the presence of SCN^- was not significantly affected by sulfite, but SCN^- increased the amount of ascorbic acid oxidized about 2-fold (compare panels B and D), indicating that SCN^- decreased the suppressive effects of sulfite on oxidation of ascorbic acid in nitrite/ascorbic acid systems.

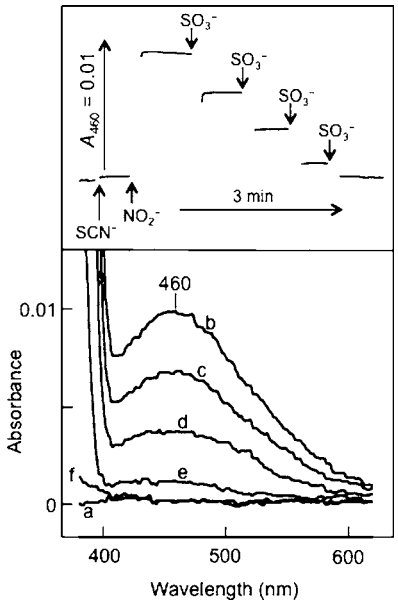

Figure 5. Consumption of ONSCN by sulfite. Reactions were run in 0.2 M KCl-HCl (pH 1.25). (Top) Time courses of absorbance changes at 460 nm. Upward arrows indicate addition of 10 mM NaSCN and 10 mM $NaNO_2$ as indicated; downward arrows indicate four successive addition of 3.3 mM Na_2SO_3. (Bottom) Sulfite-induced changes in absorption spectra of ONSCN: (a) baseline; (b) 10 mM NaSCN + 10 mM $NaNO_2$; (c) (b) + 3.3 mM Na_2SO_3; (d) (b) + 6.6 mM Na_2SO_3; (e) (b) + 9.9 mM Na_2SO_3; (f) (b) + 13.2 mM Na_2SO_3. Na_2SO_3 solution (1 M) was dissolved 1 M HCl. The pH after the addition of 13.2 mM Na_2SO_3 was 1.29.

Inhibition of NO Production by Sulfite in Nitrite/Rutin Systems. Polyphenols are present in dried fruits and wine to which sulfite is added, and rutin is a typical polyphenol in plants. Figure 11 shows the effects of sulfite on NO production in nitrite/rutin systems. The rate of nitrite-induced NO production in the presence of 1 mM rutin decreased, attaining a constant rate (trace 2 in A). The rate constant was calculated from the initial rate by postulating that 1 mol of rutin reduced 1 mol of nitrous acid to produce NO and rutin radical, which is transformed to the quinone form.[26] The value was (15.1 ± 2.0) × 10 M^{-1} min^{-1} (mean with SD, n = 4). The NO production was enhanced by 1 mM sulfite, and the rate decreased to nearly zero during incubation (trace 3 in A). Taking NO production in the presence of 1 mM sulfite (trace 1 in A) into account, the initial rate in trace 3 seemed to be the sum of those of traces 1 and 2. SCN^- did not significantly affect nitrite-induced NO production in the presence of 1 mM rutin (compare traces 2 in A and B), but was suppressed in the presence of sulfite only (compare traces 1 in A and B) and both rutin and sulfite (compare traces 3 in A and B). The amount of NO produced in nitrite/rutin systems increased and then decreased in the absence of SCN^-, whereas it decreased in the presence of SCN^- as a function of sulfite concentration (bottom panel), indicating that the effects of sulfite on nitrite/rutin systems were different from those on nitrite/ascorbic acid systems (Figure 9).

Figure 12 shows nitrite-induced absorbance changes of rutin. An absorbance decrease (352 nm) and increases (270 and 420 nm) with isosbestic points at 304 and 380 nm were observed in a nitrite/rutin system (A). The absorbance changes, which were suppressed by sulfite (B), are due to the formation of the quinone form of rutin.[26] The changes in the absorption spectra in the presence of SCN^- (absorbance increases at 280 and 320 nm and decrease at 360 nm) (C) were due to the formation of an oxathiolone derivative of rutin.[26] Sulfite also suppressed the changes in absorption spectra (D). Furthermore, 1 mM sulfite

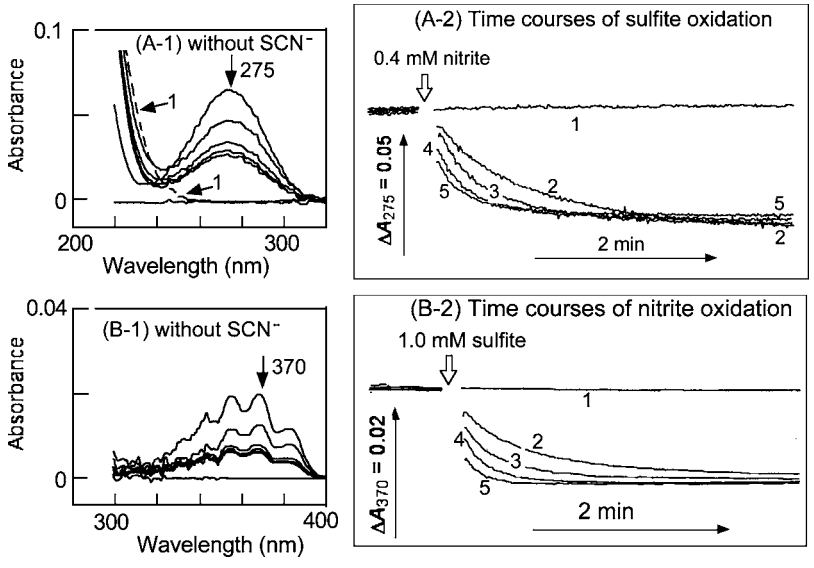

Figure 6. Decomposition of sulfite and nitrite in sulfite/nitrite systems. (A-1) Nitrite-induced absorbance decrease of sulfite. After the absorption spectrum of the reaction mixture containing 1 mM Na_2SO_3 in 50 mM KCl−HCl (pH 2.0) had been recorded, 0.4 mM $NaNO_2$ was added. Scanning was repeated every 0.92 min from 320 to 220 at 120 nm/min. Spectrum 1 with dashed line represents 0.4 mM $NaNO_2$ in 50 mM KCl−HCl (pH 2.0). (A-2) Time courses of nitrite-induced absorbance decrease of 1 mM sulfite: (1 and 2) without NaSCN; (3) 0.2 mM NaSCN; (4) 0.5 mM NaSCN; (5) 1 mM NaSCN. Downward arrow indicates (1) no addition or (2−5) addition of 0.4 mM $NaNO_2$. (B-1) Sulfite-induced absorbance decrease of nitrite. After the absorption spectrum of the reaction mixture containing 1 mM $NaNO_2$ in 50 mM KCl−HCl had been recorded, 1 mM Na_2SO_3 was added. Scanning was repeated every 0.92 min from 400 to 300 at 120 nm/min. (B-2) Time courses of sulfite-induced absorbance decrease of 1 mM nitrite: (1 and 2) without NaSCN; (3) 0.2 mM NaSCN; (4) 0.5 mM NaSCN; (5) 1 mM NaSCN. Downward arrow indicates (1) no addition or (2−5) addition of 1 mM Na_2SO_3.

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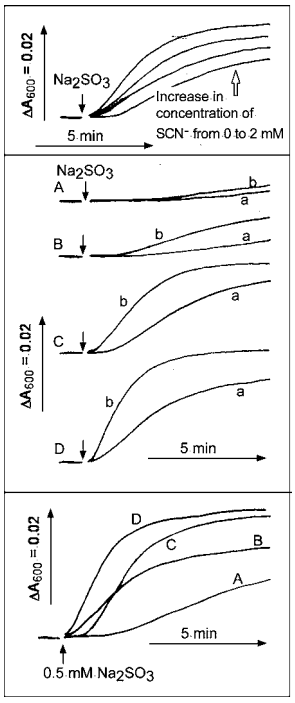

Figure 7. Formation of sulfate by sulfite/nitrite systems. (Top) SCN^--dependent enhancement. The reaction mixture contained 0.25 mM $NaNO_2$ and 0, 0.2, 0.5, or 2 mM NaSCN from the bottom to the top trace in 50 mM KCl–HCl (pH 2.0). Downward arrow indicates addition of 0.5 mM Na_2SO_3. (Middle) Effects of sulfite concentration. The reaction mixture contained 0.25 mM $NaNO_2$ and 1 mM NaSCN in 50 mM KCl–HCl (pH 2.0): (a) without NaSCN; (b) 1 mM NaSCN. Downward arrows indicate addition of (A) 0.1, (B) 0.2, (C) 0.5, and (D) 2.0 mM Na_2SO_3. (Bottom) Effects of O_2. The reaction mixture contained 0.25 mM $NaNO_2$ in 50 mM KCl–HCl (pH 2.0). Upward arrow indicates addition of 0.5 mM Na_2SO_3: (A) aerobic conditions; (B) A + 1 mM NaSCN; (C) anaerobic conditions; (D) C + 1 mM NaSCN.

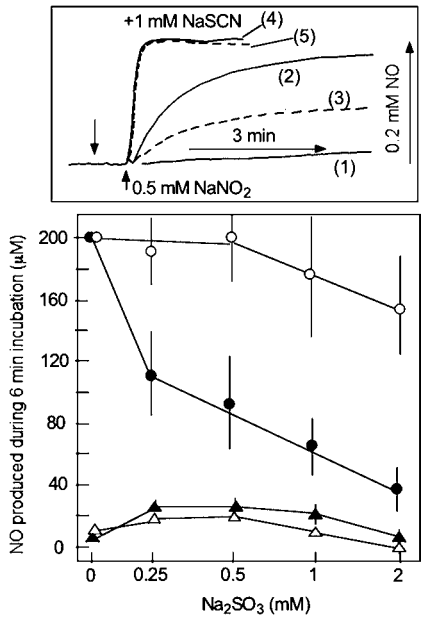

Figure 9. Effects of sulfite on NO production induced by nitrite/ascorbic acid systems. (Top) Time courses. Reactions were run in 50 mM KCl–HCl (pH 2.0): (trace 1) without ascorbic acid, sulfite, and NaSCN; (trace 2) 0.1 mM ascorbic acid; (trace 3) 0.1 mM ascorbic acid + 0.5 mM Na_2SO_3; (trace 4) 0.1 mM ascorbic acid + 1 mM NaSCN; (trace 5) trace 4 + 0.5 mM Na_2SO_3. Downward arrow indicates addition of ascorbic acid, Na_2SO_3, and NaSCN. Upward arrow indicates addition of 0.5 mM $NaNO_2$ to start reactions. (Bottom) Inhibition of NO production by sulfite. The amount of NO produced during 6 min of incubation was plotted: (▲) 0.5 mM $NaNO_2$; (△) ▲ + 1 mM NaSCN; (●) 0.5 mM $NaNO_2$ + 0.1 mM ascorbic acid; (○) ● + 1 mM NaSCN. AA, ascorbic acid.

suppressed NO production induced by 1 mM nitrite in the presence of 0.05 mM quercetin, caffeic acid, or chlorogenic acid under acidic conditions (data not shown).

DISCUSSION

The following mechanisms are possible for NO production by self-decomposition of nitrite under acidic conditions:

$$NO_2^- + H^+ \rightleftarrows HNO_2 \quad (pK_a = 3.3) \tag{2}$$

$$HNO_2 + H^+ \rightleftarrows H_2NO_2^+ \rightleftarrows NO^+ + H_2O \tag{3}$$

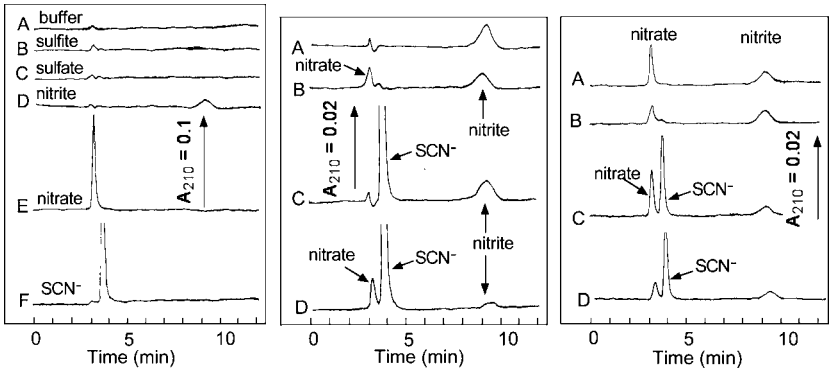

Figure 8. Sulfite-induced nitrate formation. (Left) HPLC of reagents dissolved in 50 mM KCl–HCl (pH 2.0): (A) no addition; (B) 0.5 mM Na_2SO_3; (C) 0.5 mM Na_2SO_4; (D) 0.25 mM $NaNO_2$; (E) 0.25 mM $NaNO_3$; (F) 1 mM NaSCN. (Middle) Nitrate formation and nitrite consumption. The reaction mixture contained 0.25 mM $NaNO_2$ in 50 mM KCl–HCl (pH 2.0): (A) no addition with and without 1 min of incubation; (B) 1 min of incubation after the addition of 0.5 mM Na_2SO_3; (C) with and without 1 min of incubation after the addition of 1 mM NaSCN; (D) 1 min of incubation after the addition of 0.5 mM Na_2SO_3 and 1 mM NaSCN. (Right) Effects of O_2 on nitrate formation. The reaction mixture containing 0.25 mM $NaNO_2$ and 0.5 mM Na_2SO_3 in 50 mM KCl–HCl (pH 2.0) was incubated for 2 min: (A) aerobic conditions; (B) anaerobic conditions; (C) A + 0.1 mM NaSCN; (D) B + 0.1 mM NaSCN.

dx.doi.org/10.1021/jf2049257 | J. Agric.Food Chem. 2012, 60, 1102–1112

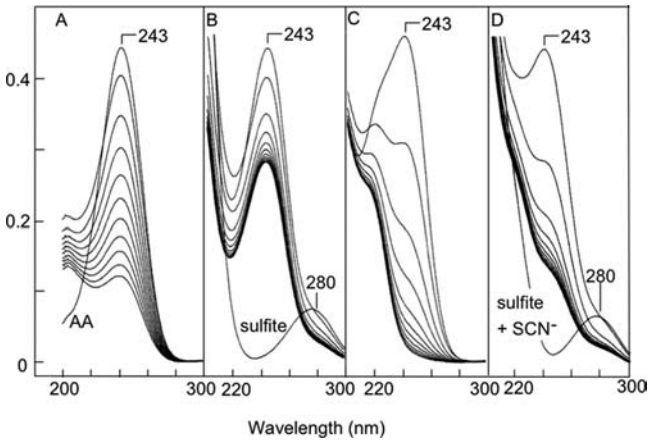

Figure 10. Effects of sulfite on nitrite-induced oxidation of ascorbic acid: (A) 0.25 mM $NaNO_2$; (B) A + 1 mM Na_2SO_3; (C) 0.25 mM $NaNO_2$ + 1 mM NaSCN; (D) C + 1 mM Na_2SO_3. The reaction mixture contained 0.1 mM ascorbic acid in 50 mM KCl–HCl (pH 2.0). AA, ascorbic acid. Scanning was repeated every minute from 300 to 200 nm.

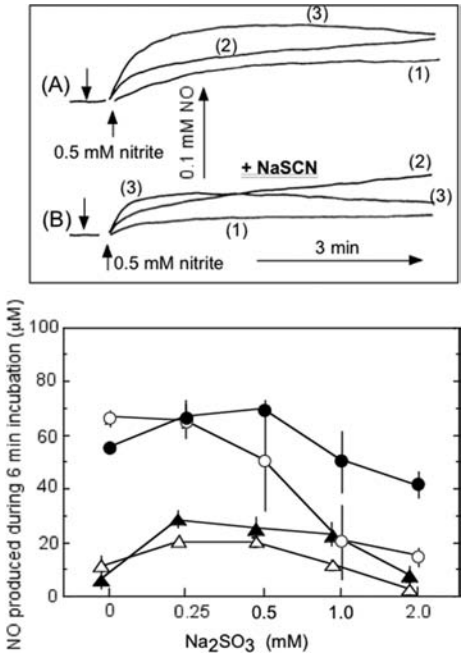

Figure 11. Effects of sulfite on NO production induced by nitrite/rutin systems. (Top) Time courses. Reactions were run in 50 mM KCl–HCl (pH 2.0): (A) without NaSCN; (B) with 1 mM NaSCN; (traces A-1 and B-1) 1 mM Na_2SO_3; (traces A-2 and B-2) 1 mM rutin; (traces A-3 and B-3) 1 mM rutin + 1 mM Na_2SO_3. Downward arrows indicate addition of rutin and/or Na_2SO_3. Upward arrows indicate addition of 0.5 mM $NaNO_2$ to start reactions. (Bottom) Effects of sulfite on NO production. Amount of NO produced during 6 min of incubation was plotted: (▲) 0.5 mM $NaNO_2$; (△) ▲ + 1 mM NaSCN; (●) 0.5 mM $NaNO_2$ + 1 mM rutin; (○) ● + 1 mM NaSCN.

$$H_2NO_2^+ + NO_2^- \rightleftarrows N_2O_3 + H_2O \quad (4)$$

or

$$2HNO_2 \rightleftarrows N_2O_3 + H_2O \quad (5)$$

$$N_2O_3 \rightleftarrows NO + NO_2 \quad (6)$$

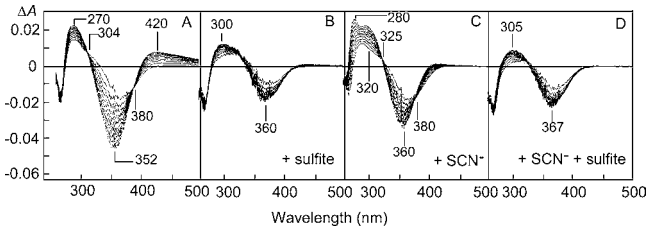

Figure 12. Effects of sulfite on nitrite-induced oxidation of rutin. The reaction mixture contained 0.5 mM rutin in 50 mM KCl–HCl (pH 2.0): (A) 0.5 mM $NaNO_2$; (B) A + 2 mM Na_2SO_3; (C) 0.5 mM $NaNO_2$ + 1 mM NaSCN; (D) C + 2 mM Na_2SO_3. After memorizing absorption spectra in the absence of nitrite, nitrite was added to record difference spectra. Scanning was repeated every minute from 500 to 260 nm.

NO production by the above reactions was slow. Sulfite enhanced NO production in the presence of nitrite (Figures 2 and 3). The following reactions are possible for the enhancement:

$$HNO_2 + HSO_3^- \rightleftarrows ONSO_3^- + H_2O \quad (7)$$

$$HNO_2 + ONSO_3^- \rightarrow 2NO + HSO_4^- \quad (c\text{-}1)$$

The rate constant of NO production via reactions 7 plus *c-1* was calculated to be $(16.2 \pm 3.9) \times 10^4$ M^{-2} min^{-1} (mean with SD; $n = 5$) using the initial rate in the presence of 0.1–0.2 mM sulfite and 1 mM nitrite. According to reactions 7 and *c-1*, the production of NO should be increased with the increase in sulfite concentration. However, the initial rate of NO production increased, attaining a constant rate, and the amount of NO produced increased and decreased as a function of sulfite concentration (Figure 3). The decrease in NO production can be explained by the enhancement of consumption of $ONSO_3^-$ and nitrite via reaction *a-1*. It is known that excess hydrogen sulfite to nitrite in concentration gives $HON(SO_3^-)_2$ mainly[27] and that $HON(SO_3^-)_2$ decomposes rapidly by H^+-catalyzed reaction to produce SO_4^{2-} and NH_4^+.[11] Reaction *b-1* will not contribute to sulfite-dependent suppression of NO production, because sulfite does not affect the decomposition of $ONSO_3^-$ via reaction *b-1*. Reaction *d-1* will not contribute significantly to the decrease in NO production because of the small rate constant (32 ± 10 M^{-1} s^{-1}).[16]

Sulfite-dependent consumption of ONSCN was confirmed in this study (Figure 5). The following reactions are possible for rapid consumption of ONSCN by sulfite:

$$ONSCN + HSO_3^- \rightarrow NO + SCN^- + HSO_3^\bullet \quad (8)$$

$$ONSCN + HSO_3^- \rightarrow ONSO_3^- + SCN^- + H^+ \quad (9)$$

According to the above reactions, enhancement of NO production by SCN^- in the presence of lower concentrations of sulfite (Figure 4) can be explained by the formation of NO by reaction 8 and the enhanced formation of $ONSO_3^-$ by reaction 9, which can react with nitrous acid by reaction *c-1*. The rate as well as the amount of NO production decreased when sulfite concentration was increased over 1 mM in the presence of SCN^- (Figure 4). The decrease can be explained by enhanced consumption of $ONSO_3^-$ and nitrite via reaction *a-1* as discussed above and the combination of NO with $HSO_3^\bullet$ that is formed by reaction 8 and other reactions (see below). The occurrence of the reaction of NO with $HSO_3^\bullet$ is possible, because NO production was studied under anaerobic conditions. SCN^--dependent enhancement of the consumption

of both nitrite and sulfite (Figure 6) supports the occurrence of reactions 1, 8, and 9 in nitrite/sulfite/SCN^- systems.

In addition to reaction 8, $HSO_3^•$ can be produced as follows using NO^+ generated by reaction 3 and NO_2 generated by reaction 6:[28]

$$HSO_3^- + NO^+ \rightarrow NO + HSO_3^• \quad (10)$$

$$HSO_3^- + NO_2 + H^+ \rightarrow HNO_2 + HSO_3^• \quad (11)$$

If $HSO_3^•$ is produced by the above reactions under aerobic conditions, the following reactions can proceed in addition to disproportionation of $HSO_3^•$ producing SO_3 and HSO_3^-:[3,29]

$$HSO_3^• + O_2 \rightarrow HSO_5^• \rightarrow SO_3 + HO_2^• \quad (12)$$

$HSO_5^•$ reacts with hydrogen sulfite to generate $HSO_3^•$, $HSO_4^•$, HSO_4^-, and $H_2S_2O_8$ (peroxodisulfonic acid).[29] $HO_2^•$ initiates radical chain reactions and reacts with NO to produce peroxynitrous acid ONOOH (pK_a = 6.8):[3]

$$HO_2^• + HSO_3^- + H^+ \rightarrow H_2O_2 + HSO_3^• \quad (13)$$

$$HO_2^• + NO \rightarrow ONOOH \quad (14)$$

H_2O_2 generated by reaction 13 oxidizes nitrous acid to ONOOH:

$$H_2O_2 + HNO_2 \rightarrow ONOOH + H_2O \quad (15)$$

ONOOH rearranges to nitric acid[3] and reacts with hydrogen sulfite to produce $HSO_3^•$ and NO_2[30] to continue oxidation of hydrogen sulfite by radical chain reactions. It has been suggested under Results that reactions other than autoxidation of NO contribute to O_2 uptake in sulfite/nitrite systems. As the mechanism of NO-independent O_2 uptake, the above radical chain reactions are possible.

The initial rate of O_2 uptake increased to attain a constant value, whereas the amount of O_2 uptake increased and decreased with the increase in sulfite concentration (Figure 3). Increases in the rate and amount of O_2 uptake are supposed to be due to the increased production of NO and $HSO_3^•$, whereas the decrease in amount is due to the increase in the rate of reaction *a-1*, which can enhance the consumption of $ONSO_3^-$ and nitrite as discussed above. The enhanced consumption of $ONSO_3^-$ and nitrite results in the suppression of production of both NO and $HSO_3^•$, which contribute to O_2 uptake, by reactions 10, 11, and *c-1*.

The rate and amount of O_2 uptake were enhanced and suppressed by SCN^-, respectively, when sulfite concentration was increased from 0.05 to 2 mM (Figure 4). According to the above discussion, the enhancement is supposed to be due to the increase in production of NO and $HSO_3^•$ via reactions 8−11 and *c-1* and the suppression due to the increase in consumption of nitrite via reactions 9 and *a-1*.

Sulfate is produced via reactions *a-1*, *b-1*, *c-1*, and *d-1* and radical chain reactions initiated by $HSO_3^•$. SCN^--dependent enhancement of sulfate formation (Figure 7) can be explained by the enhancement of production of $HSO_3^•$ and $ONSO_3^-$ by reactions 8 and 9. Faster and larger formation of sulfate under anaerobic than aerobic conditions independent of the presence and absence of SCN^- can be attributed to O_2-dependent suppression of nitrite-induced oxidation of sulfite to sulfate. O_2 can contribute to the suppression by consuming NO, a precursor of nitrite, and nitrite by reactions 14 and 15 that produce ONOOH. ONOOH is transformed to nitrate, suppressing nitrite-dependent oxidation of sulfite to sulfate. Greater formation of nitrate under aerobic than anaerobic conditions (Figure 8) supports the contribution of reactions 14 and 15 to the formation of nitrate under aerobic conditions.

Sulfite inhibited the initial rate of NO production (Figure 9) but did not inhibit the initial rate of ascorbic acid oxidation (Figure 10) in nitrite/ascorbic acid systems in the absence of SCN^-. The results suggest (i) that the inhibition of NO production was due to the competition between sulfite and ascorbic acid for nitrite and (ii) that ascorbic acid is oxidized not only by nitrous acid but also by reactive nitrogen oxide species and radicals including $HSO_3^•$, which are produced during the reactions between nitrite and sulfite. Sulfite also decreased the amount of ascorbic acid oxidized as well as NO produced. The decreases indicate effective consumption of $ONSO_3^-$ and nitrite via reaction *a-1*, decreasing the concentration of nitrite that can oxidie ascorbic acid. SCN^- decreased the inhibitory effects of sulfite. This result indicates that the reaction of ascorbic acid with ONSCN is much faster than that of hydrogen sulfite with ONSCN by reactions 8 and 9.

Sulfite increased and decreased NO production by nitrite/rutin systems at the lower and higher concentrations, respectively, in the absence of SCN^- (Figure 11). The enhancement and the suppression were supposed to be due to the additive effect of NO production by nitrite/rutin and nitrite/sulfite systems and enhanced consumption of $ONSO_3^-$ and nitrite via *a-1*, respectively. Sulfite-dependent suppression of NO production in nitrite/rutin systems was increased by SCN^-. This result suggests that the reaction of ONSCN with rutin to produce NO was slow. Sulfite-dependent inhibition of rutin oxidation in the presence and absence of SCN^- (Figure 12) indicates that sulfite can consume nitrite even when high concentrations of rutin are present in reaction mixtures.

The results in the present study suggest that when sulfite-containing foods are ingested, salivary nitrite mainly reacts with gastric ascorbic acid in the stomach because of the presence of SCN^- in the mixture of saliva and gastric juice. After the concentration of ascorbic acid is decreased, nitrite can react with sulfite and polyphenols. The reactions of polyphenols with nitrite are slowed by sulfite. If the sulfite concentration is higher than that of nitrite, almost all of the nitrite is consumed by sulfite, suppressing the production of NO. The suppression of NO production may cause various symptoms, because NO can increase stomach activities[31] and contribute to curing of some kinds of gastropathies.[32−34]

It has been reported that ingestion of wine results in the enhancement of NO production in the stomach, which is postulated to be due to the reduction of salivary nitrite by polyphenols in wine.[35,36] On the other hand, it has been reported that sulfite concentration in wine is 0.15−3.9 mM[37,38] and that nitrite concentration in saliva is 0.05−1 mM.[5,17] The data suggest that sulfite in wine suppresses NO production induced by polyphenols by enhancing the consumption of salivary nitrite via reaction *a-1* in the stomach. If the concentration of sulfite in wine is decreased, more NO can be produced in the stomach after wine is consumed. Because reactive oxygen and nitrogen oxide species are produced during the reaction between sulfite and nitrite in addition to radicals such as $HSO_3^•$, the decrease in sulfite concentration results in the slowing of production of these reactive species in the stomach after the consumption of wine.

According to the above discussion, the toxic effects of sulfite on the stomach[39] and sulfite-induced lipid peroxidation of mucosal and submucosal cells in the stomach[40] can be postulated to be due to the generation of reactive oxygen and nitrogen oxide species and radicals including $HSO_3^•$ in sulfite/nitrite systems. Furthermore, the above discussion suggests that mechanisms of sulfite-induced injuries to the stomach are different at lower and higher sulfite concentrations. Lower concentrations of sulfite can injure the stomach through the formation of reactive oxygen and nitrogen oxide species including $HSO_3^•$ without affecting NO production significantly and the higher concentration through the suppression of NO production in addition to the formation of reactive oxygen and nitrogen oxide species and radicals including $HSO_3^•$. The suppression of NO production accompanies the consumption of nitrite by the remaining sulfite in the stomach. The remaining sulfite can also injure the stomach. In this way, it seems better to avoid intake of sulfite that can decrease the production of NO, increasing the production of reactive oxygen and nitrogen oxide and radicals derived from sulfite in the stomach.

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