

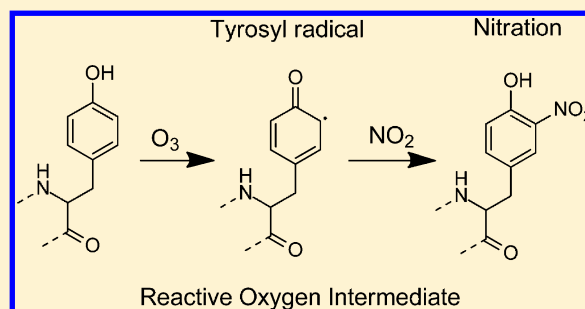
Multiphase Chemical Kinetics of the Nitration of Aerosolized Protein by Ozone and Nitrogen Dioxide

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ABSTRACT: Proteins contained in pollen and other biological particles are nitrated by ozone and nitrogen dioxide in polluted air. The nitration can enhance the allergenic potential of proteins, which may contribute to the increasing prevalence of allergic diseases. The reactive uptake of NO₂ by aerosolized protein (bovine serum albumin) was investigated in an aerosol flow tube using the short-lived radioactive tracer ¹³N. In the absence of O₃, the NO₂ uptake coefficient was below detection limit ($\gamma_{\text{NO}_2} < 10^{-6}$), but with 20–160 ppb O₃ γ_{NO_2} increased from $\sim 10^{-6}$ to $\sim 10^{-4}$. Using the kinetic multilayer model of surface and bulk chemistry (KM-SUB), the observed time and concentration dependence can be well reproduced by a multiphase chemical mechanism involving ozone-generated reactive oxygen intermediates (ROIs), but not by NO₃ radicals formed in the gas phase. Product studies show the formation of protein dimers, suggesting that the ROIs are phenoxy radical derivatives of the amino acid tyrosine (tyrosyl radicals) which are also involved in physiological protein nitration processes. Our results imply that proteins on the surface of aerosol particles undergo rapid nitration in polluted air, while the rate of nitration in bulk material may be low depending on phase state and surface-to-volume ratio.



INTRODUCTION

Airborne particulates and gaseous pollution are important environmental issues because they affect human health.¹ Pollen allergy has become a global problem and the rapid prevalence of respiratory allergic reactions induced by pollens has been on increase over the past decades.^{2,3} The reasons for the increase in allergy disease are still unclear. There are several hypotheses such as increased awareness and improved diagnostics, genetic susceptibility, psycho-social influences, nutrition, hygiene hypothesis, underlying disease, and environmental pollution.⁴ Immune responses can be affected by air pollutants including atmospheric particles, semivolatile hydrocarbons, and exhaust gases, which drive pro-allergic inflammation through the generation of oxidative stress.⁵ Air pollution associated with ozone, nitrogen dioxide, sulfur dioxide, and particulate matter can interact with pollen grains, leading to increased release of antigens characterized by modified allergenicity.^{2,6} However, the effects of air pollution on the occurrence of allergic diseases are complex and poorly understood.^{7,8}

Primary biological aerosol particles (PBAP) such as pollen, fungal spores, bacteria, biogenic polymers, and debris from larger organisms are known to influence the biosphere and public health. Their typical number and mass concentrations in the atmosphere are in the range of $10\text{--}10^4\text{ m}^{-3}$ and $10^{-3}\text{--}1\text{ }\mu\text{g m}^{-3}$, respectively.⁹ Proteins account for up to $\sim 5\%$ of urban air particulate matter and can be found in coarse biological particles such as pollen grains (diameter $>10\text{ }\mu\text{m}$). They are

also contained in the fine fraction of air particulate matter with fine fragments of pollen, microorganisms, or plant debris and mixing of proteins in rainwater with fine soil and road dust particles.^{10–13}

Recent laboratory experiments have shown that ozone and nitrogen dioxide can promote the nitration of protein molecules in polluted urban air.^{12,14,15} Indeed, nitrated proteins were detected in dust samples from various urban environments.¹² The nitration reaction leads to the addition of nitrogroups on the aromatic rings of tyrosine residues in the polypeptide chain,^{16,17} enhancing the allergenic potential of proteins.¹⁸ Inhalation and deposition of these nitrated proteins in the human respiratory tract may lead to the adverse health effects. Accumulating data suggest a strong link between protein 3-nitrotyrosine and the mechanism involved in disease development.¹⁹ This posttranslational modification provides a molecular rationale for the enhancement of allergic diseases by traffic-related air pollution in urban and rural environments, which has been observed in epidemiological studies but remains to be elucidated on a molecular level.^{12,18,20}

The purpose of this study is to investigate the kinetics and reaction mechanism of nitration of aerosolized protein by O₃ and NO₂ with atmospherically relevant concentrations. The

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NO₂ uptake by protein particles were investigated in an aerosol flow tube technique using a tracer technique based on the short-lived radioactive isotope ¹³N.²¹ Bovine serum albumin (BSA) is chosen as a well-defined model substance for proteins, which is a globular protein with a molecular mass of 67 kDa and 21 tyrosine residues per molecule. The same experiments are also conducted using deliquesced NaCl particles to evaluate the contribution of gas phase formation of NO₃ and N₂O₅ to NO₂ uptake.

MATERIALS AND METHODS

Figure 1 shows the schematics of the experimental setup. It consists of the production system of O₃ and labeled ¹³NO₂, an

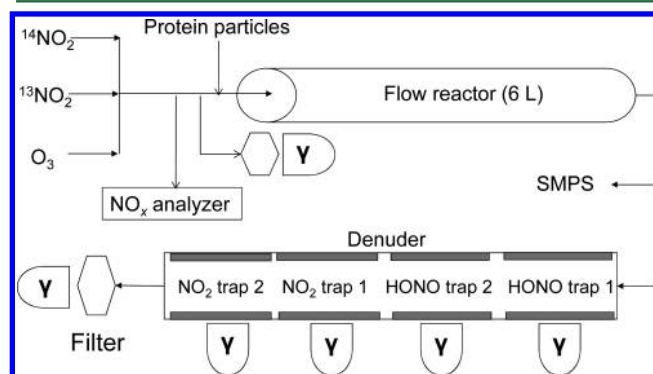


Figure 1. Scheme of the experimental setup for investigation of nitration kinetics of aerosolized protein BSA by O₃ and NO₂. The setup comprises a flow reactor, a scanning mobility particle sizer (SMPS), HONO and NO₂ traps, a particle filter, and γ -ray detectors (Y).

aerosol generation system, a flow tube reactor, and a detection system. The detail of the experimental setup was described previously.¹⁵ Briefly, ¹³NO molecules were produced through the ¹⁶O(*p*, α)¹³N nuclear reaction with 11 MeV protons in a gas target (20% O₂ in He) in continuous mode.²¹ They were converted to NO₂ by heterogeneous oxidation over firebrick granules impregnated with CrO₃.^{21,22} O₃ was generated by passing O₂/N₂ air through a quartz tube irradiated by a Xe excimer lamp. The gas flow was passed through a sand-blasted glass tube coated with sodium carbonate to remove HONO upstream of the flow tube reactor.

Aerosols were generated by nebulizing an aqueous solution of the investigated pure substance at a concentration of 0.2–0.5 g L⁻¹. The solutions were prepared by dissolving the appropriate amounts of bovine serum albumin (BSA, fraction V, >96%, Sigma) or sodium chloride in 100 mL of deionized water (18.2 M Ω cm, Milli Q plus 185, Millipore). The generated particles were dried by a diffusion dryer filled with silica gel. The dried particles were introduced into an ⁸⁵Kr source to attain an equilibrium charge distribution and then passed through a cylindrical capacitor with 3 kV potential difference between inner and outer cylinder so that only uncharged particles were passing on to the flow tube to avoid uncontrolled loss of charged particles at the wall. Then the flow was conditioned to the relative humidity of 60%. Based on hygroscopicity data of BSA,²³ the volume fraction of water in the BSA particles is estimated to be $\sim$ 10% with a pH value around $\sim$ 7 similar to the neutral aqueous solution from which the particles were generated. For NaCl particles, the flow was humidified to 75% to achieve complete deliquescence.

The aerosol surface area concentration was measured downstream of the flow tube reactor by diverting part of the flow to a scanning mobility particle sizer. The same relative humidity was maintained both in the DMA sheath flow and in the flow tube reactor by using filtered carrier gas from the flow tube as the DMA sheath flow, so that water activity and thus size of the particles remained constant during size selection in the DMA. The diameter range was 15 – 740 nm, and the observed size distribution was fitted by a single log-normal distribution to estimate the whole distribution and surface area. For BSA a typical mode diameter of the surface area distribution was $\sim$ 350 nm, and the surface concentration was in the range of 10⁻⁴ – 10⁻³ cm² cm⁻³.

The gas and aerosol flows were mixed prior to the inlet of a cylindrical 7.1 cm inner diameter and 190 cm long PFA flow tube. PFA is used to minimize the loss of NO₂ on the wall. The flow was $\sim$ 800 cm³ s⁻¹ in the reactor and the maximum residence time was $\sim$ 10 min. Kinetic experiments usually involved measurements of NO₂ uptake at five different contact times in the range of 2–10 min adjusted by moving the inlet along the PFA tube. The gas phase concentrations of NO₂ and O₃ were in the range of 5–110 ppb and 0–160 ppb, respectively. The pressure of the flow reactor was kept constant at $\sim$ 980 hPa and the temperature was kept at 296 K by efficient laboratory air conditioning.

After passing through the flow tube, the gas and aerosol flow entered a narrow parallel-plate diffusion denuder train coated to selectively absorb gas phase HONO and NO₂.^{21,24} For HONO absorption, the first and second sections of the denuder train were coated with sulphanilamide (SA). In this study, however, no HONO formation was observed. For absorption of NO₂, the third and fourth section of the denuder train were coated with a 1:1 mixture of N-(1-naphthyl) ethylenediamine dihydrochloride (NDA) and KOH. The aerosol particles have a small diffusivity, pass the denuder with close to 100% efficiency,^{21,25} and were retained in the glass fiber filter. The gamma-radiation detectors were attached to each denuder section and to the filter to detect the amount of gamma quanta emitted after the decay of ¹³N, corresponding to the amount of trapped ¹³N-containing molecules. Note that for the particle signal only strongly bound (chemisorbed) ¹³N species are detected.^{21,24}

Protein product studies were performed with an HPLC-chip-MS/MS system (Agilent Technologies).¹⁶ The electrospray ionization (ESI) Q-TOF instrument was operated in the positive ionization mode (ESI+) with an ionization voltage of 1750 V and a fragmentor voltage of 175 V at 300 °C. The MS mode was used and the selected *m/z* ranges were 200–3000 Da. Protein identification and deconvolution was performed with Agilent MassHunter Bioconfirm Software (version B.03) using the Large Molecular Feature Extraction (MFE) algorithm. The sequence matching was performed with a mass accuracy of $\pm$ 30 ppm.

RESULTS AND DISCUSSION

Observed NO₂ Uptake. Protein BSA and deliquesced NaCl particles were exposed to NO₂ (6, 30 ppb) under humid conditions (25, 60% RH). Uptake of NO₂ was not observed in the investigated range of NO₂ concentration and relative humidity. The estimated upper limit of uptake coefficient of NO₂ (γ_{NO_2}) for BSA particles is $\sim$ 1 $\times$ 10⁻⁶. This indicates NO₂ itself cannot nitrate protein efficiently, which is consistent with previous studies.^{12,15}

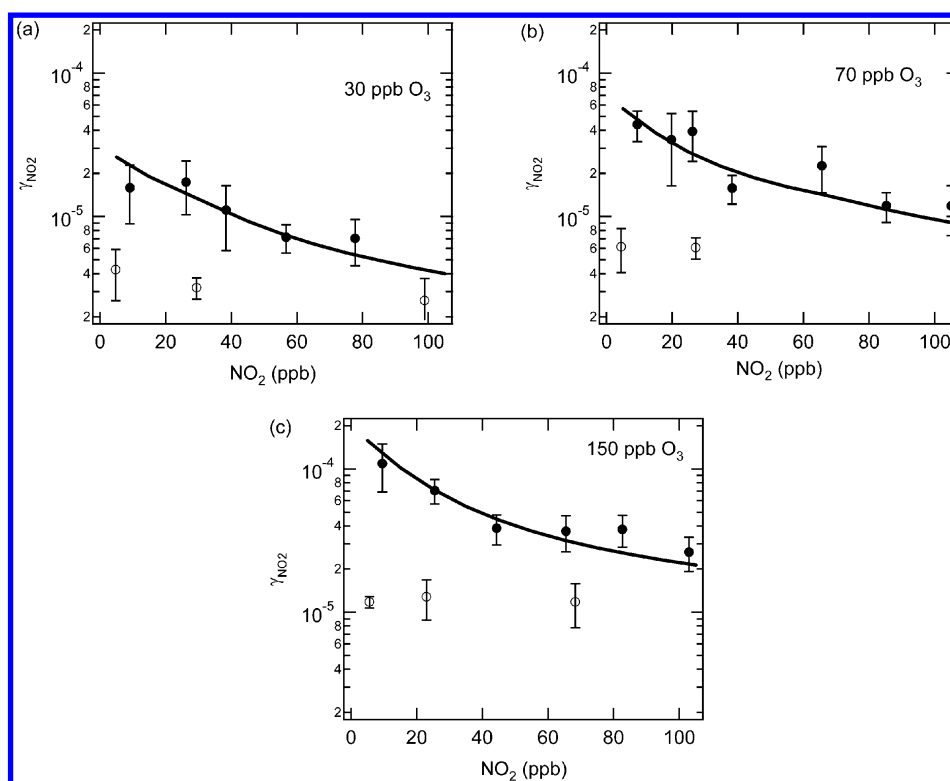


Figure 2. Uptake coefficients of NO_2 (γ_{NO_2}) by BSA (circle) and deliquesced NaCl (open circle) particles under 60% RH as a function of NO_2 gas phase concentration with fixed O_3 gas phase concentration of (a) 30 ppb, (b) 70 ppb, and (c) 150 ppb. The lines are modeled by KM-SUB including the formation of reactive oxygen intermediates (ROIs).

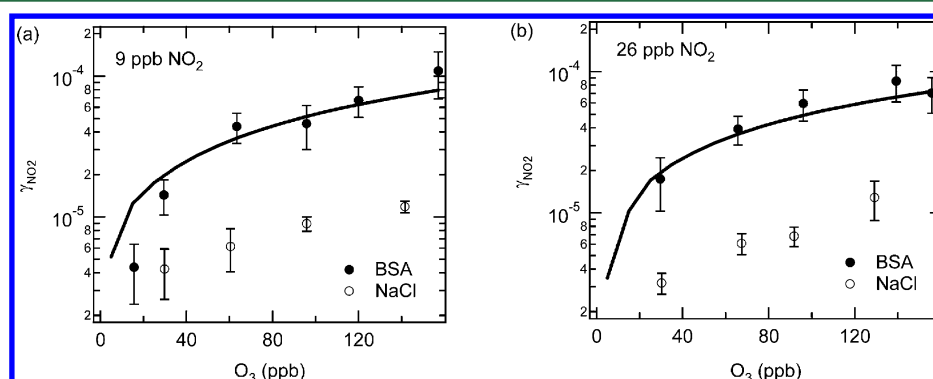


Figure 3. Uptake coefficients of NO_2 (γ_{NO_2}) by BSA (circle) and deliquesced NaCl (open circle) particles under 60% RH as a function of O_3 gas phase concentration with fixed NO_2 gas phase concentration of (a) 9 ppb and (b) 26 ppb. The lines are modeled by KM-SUB including the formation of reactive oxygen intermediates (ROIs).

NO_2 uptake was also not observed on deliquesced NaCl particles with an estimated upper limit of γ_{NO_2} of $\sim 1 \times 10^{-7}$. Abbatt and Waschewsky²⁶ reported an upper limit of γ_{NO_2} by liquid NaCl aerosol of $\sim 1 \times 10^{-4}$. Our results are consistent with their results and provide an improved upper limit. This is in line with some earlier studies indicating that the bulk aqueous phase reaction of NO_2 with chloride to form NOCl and nitrate is very slow.^{27–29} It however also contrasts with other experiments, though performed at significantly higher concentrations of NO_2 , where uptake coefficients of up to 10^{-4} have been reported.^{30,31} Yabushita et al.³¹ propose a surface complex $\text{Cl}^- \times \text{NO}_2^{\bullet-}$ to play a significant role at millimolar chloride concentration, that is, for more dilute solutions than the deliquesced particles of the present study.

Figure 2 shows γ_{NO_2} on BSA and deliquesced NaCl particles as a function of NO_2 gas phase concentration at 60% RH and at different O_3 gas phase concentrations of (a) 30 ppb, (b) 70 ppb, and (c) 150 ppb. Depending on O_3 concentration, γ_{NO_2} is in the range of 10^{-5} – 10^{-4} . In agreement with related earlier investigations,¹⁵ γ_{NO_2} stays almost constant up to ~ 30 ppb NO_2 and decreases gradually as the NO_2 concentration increases further. In contrast, γ_{NO_2} on deliquesced NaCl particles are 1 order of magnitude lower than that by BSA and showed only a rather insignificant dependence on the NO_2 concentration.

Figure 3 shows γ_{NO_2} as a function of O_3 concentration at 60% RH and at NO_2 concentrations of (a) 9 ppb and (b) 26 ppb. γ_{NO_2} shows pronounced nonlinear increases with increasing O_3 concentration. For example, at 9 ppb NO_2 γ_{NO_2} on BSA is 4.4×10^{-6} at 16 ppb O_3 , which is enhanced by a factor of ~ 30 to 1.0

$\times 10^{-4}$ at 158 ppb O_3 . γ_{NO_2} on deliquesced NaCl is 1 order of magnitude smaller, which is likely to be attributed to the formation of gas phase NO_3 and N_2O_5 , as neither O_3 nor NO_2 is expected to rapidly react with deliquesced NaCl. The impact of formation of gas phase NO_3 and N_2O_5 on NO_2 uptake is investigated by kinetic modeling as discussed in detail below.

Formation of NO_3 and N_2O_5 . NO_2 can be oxidized by O_3 to form highly reactive NO_3 radicals in the gas phase, which can react further with NO_2 to form N_2O_5 . NO_3 radicals can directly react with liquid and solid organic surfaces exhibiting uptake coefficients in the range of $10^{-3} - 0.1$.^{32–35} N_2O_5 can undergo heterogeneous hydrolysis to form HNO_3 on humid surfaces. Extensive laboratory investigations of the reactive uptake coefficients of N_2O_5 on aqueous inorganic particles showed reactive uptake coefficients between $10^{-2} - 0.15$.^{36–38}

To evaluate the potential influence of gas phase NO_3 and N_2O_5 on the experimental results, the temporal evolution of gas phase species and uptake of NO_3 and N_2O_5 was estimated using a kinetic model.³⁹ The gas phase reactions considered in the model are listed in Table 1 with gas phase reaction rate

Table 1. Gas Phase Reactions (GPR) Included in the Kinetic Box Model

	gas phase reaction	rate coefficient (296 K)
GPR1	$NO_2 + O_3 \rightarrow NO_3 + O_2$	$3.2 \times 10^{-17} \text{ cm}^3 \text{ s}^{-1}$
GPR2	$NO_3 + h\nu \rightarrow NO + O_2$	$0 - 0.016 \text{ s}^{-1}$
GPR3	$NO_3 + h\nu \rightarrow NO_2 + O$	$0 - 0.19 \text{ s}^{-1}$
GPR4	$NO + O_3 \rightarrow NO_2 + O_2$	$1.8 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$
GPR5	$NO + NO_3 \rightarrow 2NO_2$	$2.6 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1}$
GPR6	$NO_2 + NO_3 + M \rightleftharpoons N_2O_5 + M$	$K_{eq} = 2.9 \times 10^{-11} \text{ cm}^3$
GPR7	$NO_3 + M \rightarrow NO + O_2 + M$	$3.0 \times 10^{-3} \text{ s}^{-1}$

coefficients.⁴⁰ The photolysis rate of NO_3 (GPR2 and GPR3) is hard to determine as the reported values are under sunlight conditions, whereas the experiments were done under undefined light intensity in the laboratory. For simplicity the photolysis rates were assumed to be 0, which can lead to an overestimation of the NO_3 concentration. The reactive uptake of NO_3 and N_2O_5 by particles was described by elementary gas-surface reaction probabilities (γ_{gsr}).^{39,41} To estimate the possible maximum contribution of NO_3 and N_2O_5 to the apparent uptake of NO_2 (labeled by ^{13}N), $\gamma_{gsr} = 1$ was assumed for NO_3 and N_2O_5 and wall losses of NO_3 and N_2O_5 in the flow tube wall were neglected.

The model results show that the steady-state condition for gas phase concentrations is achieved within ~ 1 min in the flow reactor. The concentrations of O_3 and NO_2 showed only a

slight decrease ($<3\%$) and remained practically constant. The NO_3 and N_2O_5 gas phase concentrations were of the order of ~ 0.1 and ~ 1 ppt, respectively, whereas the NO concentration remained low around ~ 0.01 ppt. Figure 4 shows the measured and modeled temporal evolution of the fraction of N ($[N \text{ on particle}]/[NO_2]_g$) on (a) BSA particles at 121 ppb O_3 and 10 ppb NO_2 and (b) deliquesced NaCl particles at 142 ppb O_3 and 6 ppb NO_2 . As shown by the dotted line in Figure 4(a) the model underestimates the observed uptake of NO_2 by BSA significantly. NO_3 and N_2O_5 uptake could explain only $\sim 30\%$ of the observed uptake, which is certainly a conservative upper limit. Therefore, gas phase NO_3 and N_2O_5 are not the main contributors to the observed reactive nitrogen uptake by BSA.

γ_{NO_3} and $\gamma_{N_2O_5}$ on dilute solution of NaCl is reported to be 6×10^{-3} ⁴² and 0.01,⁴³ respectively. Using these values the model can successfully reproduce the observed NO_2 uptake by deliquesced NaCl particles as shown by the dashed line Figure 4(b). The dotted line is modeled with $\gamma_{NO_3} = \gamma_{N_2O_5} = 1$, showing the possible maximum contribution of NO_3 and N_2O_5 to the NO_2 uptake. These results demonstrate that gas phase NO_3 and N_2O_5 are the main contributors to NO_2 uptake by deliquesced NaCl particles.

Formation of Reactive Oxygen Intermediates (ROI). Shiraiwa et al.¹⁵ observed an efficient uptake of NO_2 with $\gamma_{NO_2} \approx 10^{-4}$ when the BSA particles were pretreated with O_3 (97 ppb) and then exposed to NO_2 in absence of O_3 . The high reactivity of NO_2 with O_3 pretreated particles can be explained by a nitration reaction involving ozone-generated reactive oxygen intermediates (ROIs), but not by alternative pathways (reaction with NO_3 and N_2O_5 formed in the gas phase). The chemical identity of the ROI is most likely a phenoxy radical derivative of tyrosine as discussed below.

Here we model the formation of reactive oxygen intermediates (ROIs) using the kinetic multilayer model for aerosol surface and bulk chemistry (KM-SUB).⁴⁴ KM-SUB consists of multiple model compartments and layers, respectively: gas phase, near-surface gas phase, sorption layer, quasi-static surface layer, and a number of n bulk layers. KM-SUB explicitly resolves surface-bulk exchange, bulk diffusion and chemical reactions from the gas-particle interface to the particle core, resolving concentration gradients and diffusion throughout the particle bulk. In the numerical simulations presented in this study, the number of bulk layers was set to $n = 100$. Test calculations using smaller or higher values of n gave almost identical results. The temporal evolution of the particle surface and bulk composition and γ were modeled by

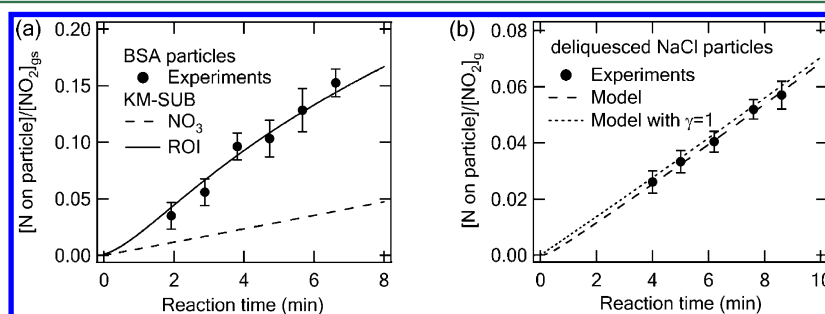
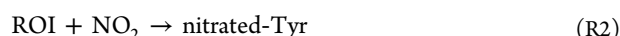


Figure 4. Measured fraction of N ($[N \text{ on particle}]/[NO_2]_g$) on (a) BSA particles at 121 ppb O_3 and 10 ppb NO_2 and (b) deliquesced NaCl particles at 142 ppb O_3 and 6 ppb NO_2 as function of reaction time. The solid line is modeled considering the formation of ROIs and the dashed lines are modeled considering the contribution from gas phase NO_3 and N_2O_5 formation and their uptake to particles.

numerically solving the differential equations for the mass balance of each model compartment.

The following chemical reactions are considered at the surface and in the bulk of the protein particles.



Reaction rates are calculated assuming that reactions proceed with second-order rate dependencies on the concentrations within each bulk layer. Here we assume that a quarter of tyrosine amino acid residues in BSA is reactive toward O_3 ,^{16,45} resulting in the initial surface and bulk concentrations of reactive tyrosine of $2.8 \times 10^{13} \text{ cm}^{-2}$ and $6.5 \times 10^{19} \text{ cm}^{-3}$, respectively. The second-order surface and bulk reaction rates for R1 are adopted from estimation by Shiraiwa et al.⁴⁵ Note that R1 may also lead to the formation of O_3^- radicals which can be rapidly converted into OH radicals.⁴⁶ The secondary chemistry potentially related to this process goes beyond the scope of this study, but we intend to investigate this aspect in follow-up studies.

The additional kinetic parameters for the model simulations include surface accommodation coefficients ($\alpha_{s,0}$), desorption lifetimes (τ_d), Henry's law coefficients ($K_{\text{sol,cc}}$), and bulk diffusion coefficients (D_b) for O_3 and NO_2 . D_b for protein is assumed to be $10^{-20} \text{ cm}^2 \text{ s}^{-1}$.⁴⁵ The initial estimates of these parameters for O_3 are taken from our previous study⁴⁵ and we assume that the parameters of NO_2 are similar to those of O_3 . In addition to these parameters the reaction rate coefficients for R2 are systematically and iteratively varied using Matlab software to find a best fit to the experimental data, resulting in the kinetic parameters listed in Table 2.

Table 2. Kinetic Parameters for the Interaction Between O_3 , NO_2 , and Protein (Tyrosine Residues)

parameter ^a (Unit)	O_3	NO_2
$\alpha_{s,0}$	1	1
τ_d (s)	$(1-5) \times 10^{-8}$	$(1-5) \times 10^{-8}$
k_{SLR} ($\text{cm}^2 \text{ s}^{-1}$)	10^{-11} (R1)	10^{-10} (R2)
k_{BR} ($\text{cm}^3 \text{ s}^{-1}$)	1.6×10^{-15} (R1)	10^{-12} (R2)
$K_{\text{sol,cc}}$ ($\text{mol cm}^{-3} \text{ atm}^{-1}$)	10^{-3}	10^{-3}
D_b ($\text{cm}^2 \text{ s}^{-1}$)	10^{-9}	10^{-9}

^a $\alpha_{s,0}$: surface accommodation coefficient on adsorbate-free substrate, τ_d : desorption lifetime, k_{SLR} , k_{BR} : second-order rate coefficients of surface (k_{SLR}) and bulk (k_{BR}) reaction between O_3 and Tyr (R1), and between ROI and NO_2 (R2), $K_{\text{sol,cc}}$: Henry's law coefficient, D_b : bulk diffusion coefficients.

As shown with the solid line in Figure 4(a), KM-SUB reproduces the temporal evolution of the fraction of N ($[\text{N on particle}]/[\text{NO}_2]_g$) on BSA particles well. Moreover, KM-SUB can reproduce the dependence on both O_3 and NO_2 gas phase concentration very well as shown with solid lines in Figures 2 and 3. R1 is found to be orders of magnitude slower than R2, indicating that the reaction of protein with O_3 and formation of ROI is the rate-limiting step.

Figure 5 shows an exemplary KM-SUB simulation result at 100 ppb O_3 and 100 ppb NO_2 with 350 nm diameter particle. γ_{O_3} is constant at 2×10^{-5} up to ~ 10 s and afterward started decreasing due to the consumption of reactive tyrosine at the surface as shown in Figure 5(b). The surface concentration of ROI increases to $\sim 10^{11} \text{ cm}^{-2}$ and then decreases due to reaction with NO_2 . γ_{NO_2} is initially smaller than γ_{O_3} because the ROI concentration is initially low, but afterward converges to γ_{O_3} , indicating NO_2 uptake is limited by O_3 uptake, namely formation of ROI via R1. As shown in Figure 5(b), the surface concentrations of O_3 and NO_2 stay constant at a low level of 10^9 cm^{-2} .

Figure 6 shows the radial profiles of bulk concentration of each species simulated by KM-SUB. The y-axis indicates the radial distance from the bottom of the protein coating (r) normalized by the particle radius (r_p), ranging from the bottom ($r/r_p \approx 0$) to the surface ($r/r_p \approx 1$). The isolines show the radial distribution and temporal evolution of each species in the protein matrix. Due to the low diffusivity and reactive consumption, ozone initially exhibits a steep concentration gradient near the surface ($r/r_p > 0.95$), while the underlying bulk material remains essentially ozone-free. After ~ 10 s ozone begins to diffuse further into the bulk and the reactive tyrosines are depleted near the surface showing a strong concentration gradient (Figure 6(c)). The reaction rate of R1 is initially high near the surface and the reaction front proceeds further into the particle bulk ($r/r_p > 0.95$) (Figure 6(f)). NO_2 initially diffuses through the particle because ROI concentration is very low. After substantial ROI is produced in the bulk as well as shown in Figure 6(d), NO_2 is consumed quickly by reacting with the ROIs leading to a decrease of the ROI bulk concentration and an increase of nitrated tyrosine bulk concentration (Figure 6(e)). The production of nitrated tyrosine is confined to the near-surface bulk ($r/r_p > 0.95$) because the formation of ROI is also limited in the near-surface bulk due to the low bulk diffusivity of O_3 . The production rate profile of nitrated tyrosine looks very similar to Figure 6(f), as R1 is the rate-limiting step.

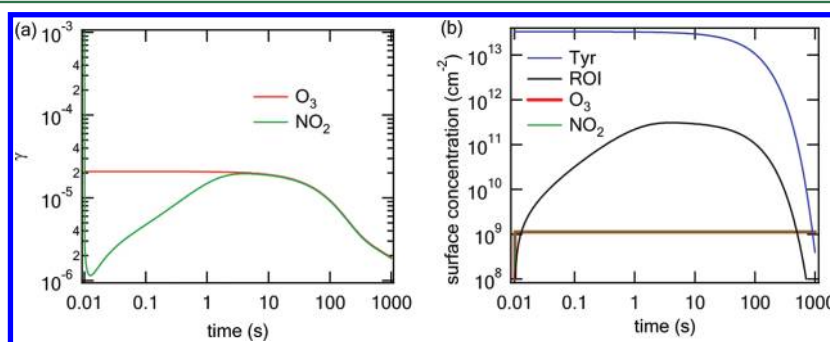


Figure 5. Kinetic model results for O_3 and NO_2 uptake by protein BSA at 100 ppb O_3 and 100 ppb NO_2 . (a) Uptake coefficients of O_3 and NO_2 . (b) Surface concentrations of reactive tyrosine, reactive oxygen intermediate (ROI), O_3 and NO_2 .

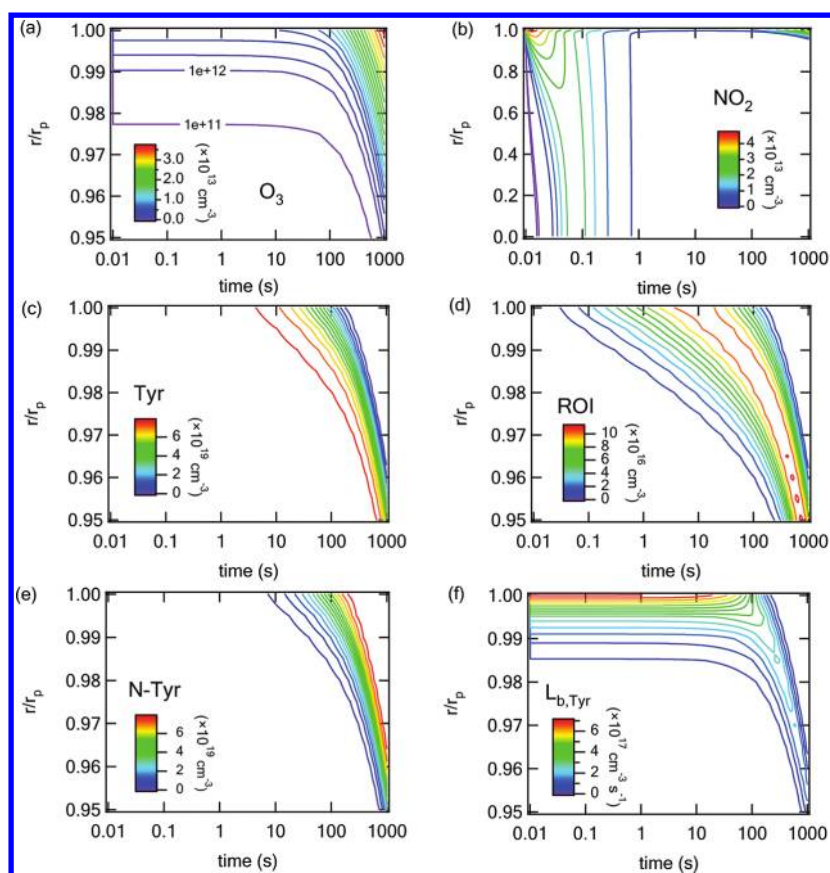


Figure 6. Radial profiles of bulk concentration of (a) O_3 , (b) NO_2 , (c) reactive tyrosine (ROI) and (d) ROI and (e) nitrated tyrosine (N-Tyr) calculated by KM-SUB at 100 ppb O_3 and 100 ppb NO_2 . (f) Loss rate profiles of reactive tyrosine due to R1. r/r_p is the distance from the particle center normalized by the particle radius ($r/r_p = 1$ at the surface).

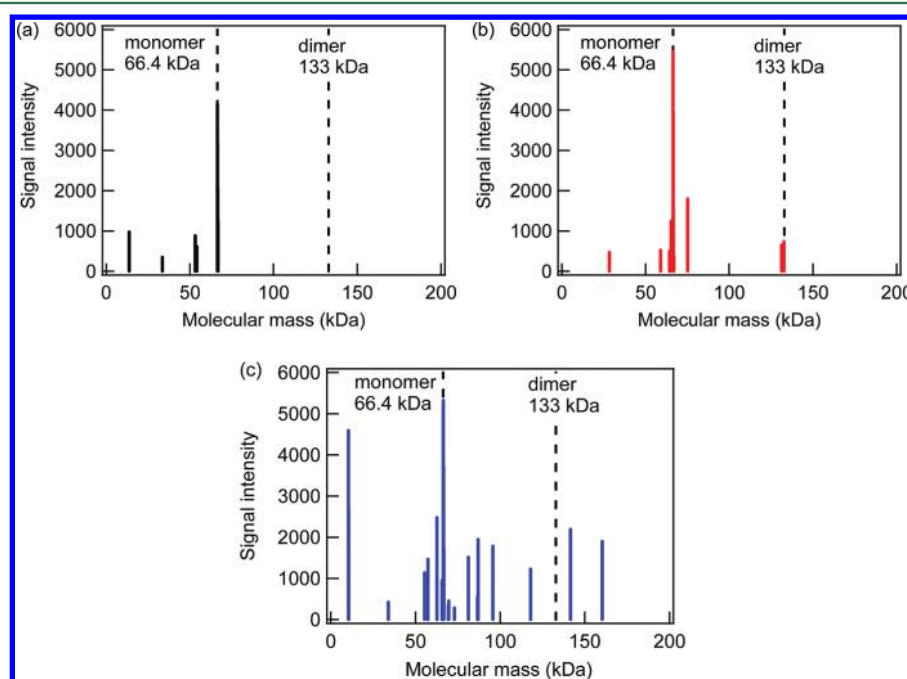


Figure 7. Mass spectra of (a) native BSA and BSA exposed to O_3 at (b) dry and (c) wet conditions (95% RH).

Shiraiwa et al.¹⁵ had speculated that the ROI may be a phenoxy radical derivative of tyrosine (tyrosyl radical). Phenoxy radicals are well established intermediates in the nitration of phenolic compounds by NO_2 ,^{47–49} and they can be efficiently

stabilized in condensed phases,⁵⁰ which is consistent with the long lifetime of the ROI. Stable ozonolysis products like dihydroxy aromatics^{51,52} are unlikely to act as intermediates, because they typically exhibit low reactivity toward NO_2 (γ_{NO_2}

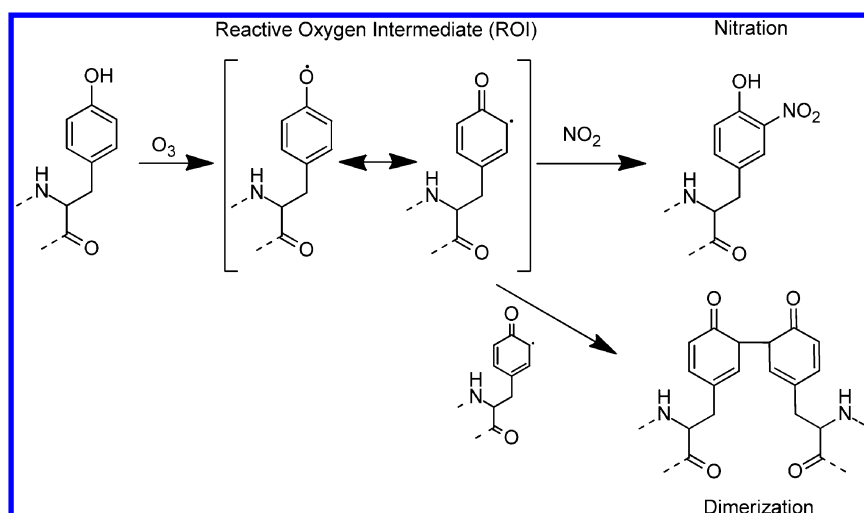


Figure 8. Schematics of reaction pathways of protein BSA and ozone and nitrogen dioxide.

$\sim 10^{-6}$).²⁴ In the course of protein nitration by reactive oxygen and nitrogen species (ROS/RNS) under physiologically relevant conditions, tyrosyl radicals are formed and can react with each other leading to the formation of protein dimers.⁵³ To test if the protein nitration by gaseous O_3 and NO_2 under environmental conditions also involves tyrosyl radicals, we conducted product studies with ozonized protein. BSA was exposed to 140 ppb O_3 for 2 h both at dry and 95% RH using a coated wall flow tube technique.⁴⁵ Protein samples were extracted from the tube using Milli-Q water for product analysis. The extracted samples were freeze-dried and analyzed by HPLC-chip-MS/MS.

Figure 7 shows the mass spectra of native and ozonized BSA. The molecular mass of native BSA is 66.4 kDa, and products with higher molecular mass could be detected after ozone exposure. Under dry conditions we found BSA dimers with a molecular mass of 133 kDa. After O_3 exposure at 95% RH we found also other products with intermediate and higher molecular masses, which can be explained by secondary chemistry involving the incorporation of oxygen atoms,^{51,52} partial decomposition of protein molecules and agglomeration of decomposition products. The chemical transformation at 95% RH was more pronounced because O_3 can diffuse and react faster in the semisolid humid protein than in the solid dry protein.⁴⁵ Moreover, water may influence the reaction pathways of protein ozonation and decomposition. Further studies will be required to fully unravel the chemical mechanism protein ozonation and nitration, but the observation of protein dimers supports the hypothesis that the ROIs involved in protein nitration are tyrosyl radicals. See Figure 8 for a schematic of the reaction pathway.

In summary, our experimental data and model results suggest that the nitration of proteins by O_3 and NO_2 proceeds through a chemical mechanism involving two steps. The first and rate-limiting step is the reaction of protein with ozone forming phenoxyl radical derivatives (tyrosyl radicals) as long-lived reactive oxygen intermediates (ROIs). The second step is a fast reaction of the ROIs with NO_2 forming nitrated tyrosine residues in the protein. In the absence of NO_2 , the ROIs can react with each other forming protein dimers. The tyrosine residues near the surface of protein particles are expected to be efficiently nitrated on a time scale of seconds to minutes under ambient atmospheric conditions, especially in polluted air with

high ozone concentration. Further nitration of tyrosine residues in the bulk of protein particles is kinetically limited by the diffusivity of O_3 and NO_2 ($\sim 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ in semisolid protein at $\sim 60\%$ RH). Thus, the allergenic potential of proteins on the surface of aerosol particles can be rapidly enhanced by nitration in polluted air,^{15,18} while the rate and degree of nitration in bulk material may be low depending on the phase state and surface-to-volume ratio of the particles.

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Notes

The authors declare no competing financial interest.

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