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Comparison of nitrate formation mechanisms from free amino acids and amines during ozonation: a computational study†

Shuning Yin, Qunfang Shen, Yong Dong Liu * and Rugang Zhong

Nitrate as a potential surrogate parameter for abatement of micropollutants, oxidant exposure, and characterizing oxidant-reactive DON during ozonation has attracted extensive attention, however, understanding of its formation mechanisms is still limited. In this study, nitrate formation mechanisms from amino acids (AAs) and amines during ozonation were investigated by the DFT method. The results indicate that *N*-ozonation initially occurs to produce competitive nitroso- and *N*,*N*-dihydroxy intermediates, and the former is preferred for both AAs and primary amines. Then, oxime and nitroalkane are generated during further ozonation, which are the important last intermediate products for nitrate formation from the respective AAs and amines. Moreover, the ozonation of the above important intermediates is the nitrate yield-controlling step, where the relatively higher reactivity of the C=N moiety in the oxime compared to the general C_α atom in the nitroalkane explains why the nitrate yields of most AAs are higher than those from general amines, and it is the larger number of released C_α^- anions, which are the real reaction sites attacked by ozone, that leads to the higher nitrate yield for nitroalkane with an electron-withdrawing group bound to the C_α atom. The good relationship between nitrate yields and activation free energies of the rate-limiting step ($\Delta G^{\neq}_{\text{rls}}$) and nitrate yield-controlling step ($\Delta G^{\neq}_{\text{nycs}}$) for the respective AAs and amines verifies the reliability of the proposed mechanisms. Additionally, the bond dissociation energy of C_α–H in the nitroalkanes formed from amines was found to be a good parameter to evaluate the reactivity of the amines. The findings here are helpful for further understanding nitrate formation mechanisms and predicting nitrate precursors during ozonation.

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Environmental significance

Nitrate as a potential surrogate parameter for abatement of micropollutants, oxidant exposure, and characterizing oxidant-reactive dissolved organic nitrogen (DON) during ozonation has attracted extensive attention. However, understanding of its formation mechanisms is still limited. In this study, nitrate formation mechanisms from amino acids (AAs) and amines during ozonation were investigated using quantum chemical calculations. It was found that oxime and nitroalkane are the important last intermediate products for nitrate formation from the respective AAs and amines. Moreover, it is the difference in reactivity between the C=N moiety in the oxime and the C_α atom in the nitroalkane in the ozonation that leads to the different nitrate yields of AAs and amines. Moreover, the bond dissociation energy of C_α–H in the nitroalkanes formed from amines was found to be a good parameter to evaluate the reactivity of various amines and to predict their nitrate molar yields.

1 Introduction

As a green and efficient disinfectant, ozone can inactivate pathogenic microorganisms and reduce micropollutants and the formation of disinfection by-products (DBPs).[1–12] However, nitrate as the endpoint product of ozonation of dissolved organic nitrogen (DON) has been found in epidemiological studies to be associated with methemoglobinemia, colorectal cancer, thyroid cancer, neuroepithelial brain tumours, and adverse health outcomes.[13–18] Recent research studies have demonstrated that nitrate could be a potential surrogate parameter for abatement of micropollutants, oxidant exposure, and characterizing oxidant-reactive DON during ozonation.[19] Accordingly, nitrate has attracted extensive attention for decades in the environmental field especially in the ozonation of drinking water.

As is known, DON compounds especially free amino acids (AAs) and amines are nitrate precursors in water during ozonation.[19–21] Studies have shown that various DON compounds have different nitrate formation potentials, and even the same precursor exhibits different nitrate molar yields

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Beijing Key Laboratory of Environmental and Viral Oncology, College of Life Science & Bioengineering, Beijing University of Technology, Beijing 100124, China. E-mail: ydliu@bjut.edu.cn; Fax: +86-10-6739-2001

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under different reaction conditions such as varying $[O_3]$/[reactant] (O/N) ratio, pH, and reaction time.[7,22–26] For free AAs, as shown in Table 1, at the O/N ratio of 5, the nitrate molar yields in the ozonation of serine (Ser) and glycine (Gly) reach 55–57%, those of glutamic acid (Glu), alanine (Ala), phenylalanine (Phe), and aspartic acid (Asp) are 40–54%, and that of cysteine (Cys) is 30%.[22] This indicates that the nitrate molar yields of free AAs are ≥30%. Regarding amines, Table 1 shows that the nitrate yields of primary amines such as 4-nitrobenzylamine, 4-chlorobenzylamine, benzylamine (BA), and ethanolamine are 22–72% and those of ethylamine (EA) and β-alanine are 8–9%.[22] For secondary and tertiary amines, their nitrate molar yields are generally below 10% except for *N*-methyl-benzylamine (19%).[22] These results indicate that most free AAs have relatively higher nitrate molar yields than primary amines, and most secondary and tertiary amines have the lowest nitrate yields among AAs and amines. What are the fundamental reasons for such large differences in nitrate molar yields between AAs and amines under ozonation? Are there any parameters of the precursors or intermediates that can represent the reactivity of the precursors and the nitrate yield?

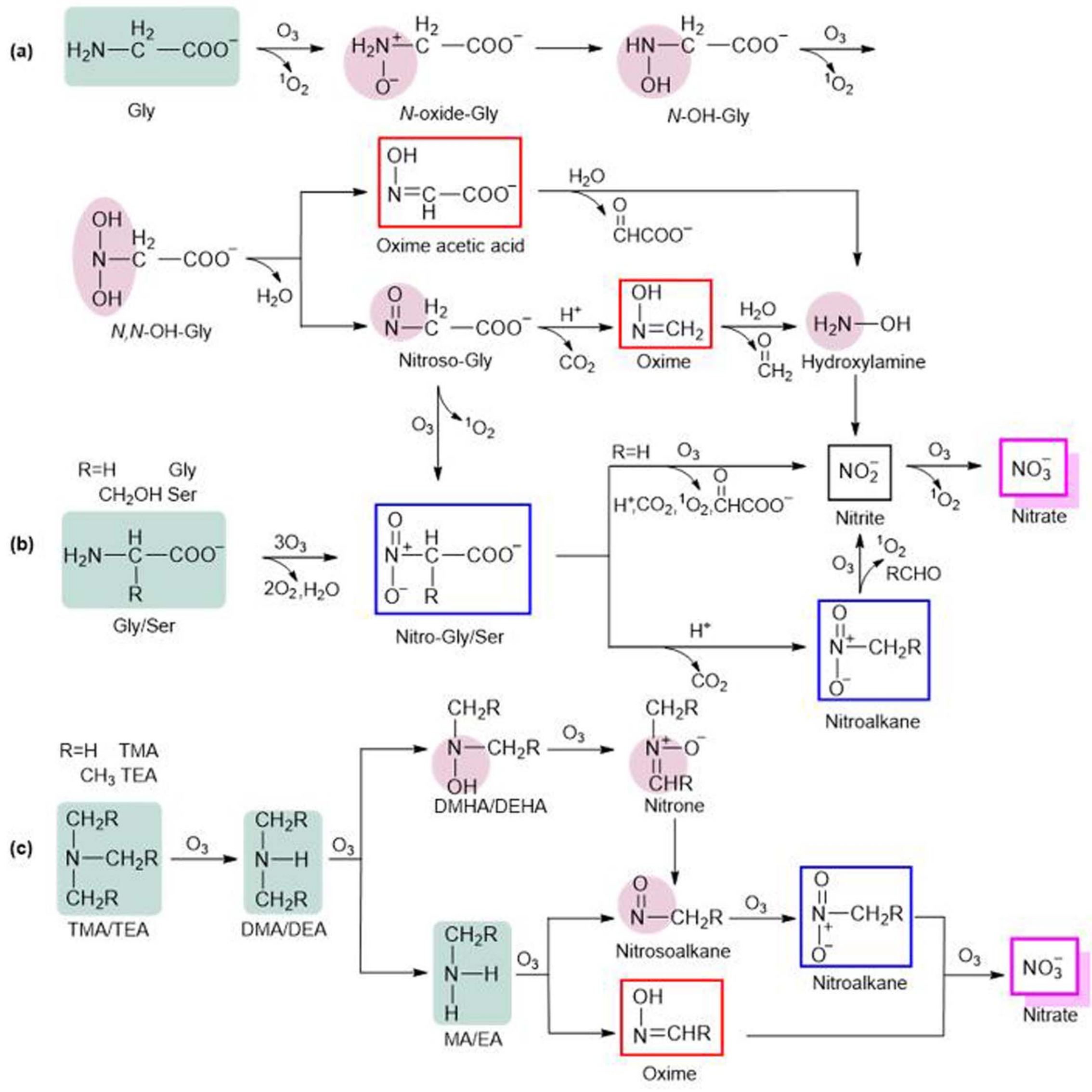
Regarding nitrate formation mechanisms, progress has been made for the simple AAs and amines. For glycine (Gly), as shown in Scheme 1(a), previous studies proposed that the initial attack of ozone takes place at the nitrogen of Gly, and then *N*-monohydroxyl-Gly (*N*-OH-Gly) and *N*-dihydroxyl-Gly (*N*,*N*-OH-Gly) form successively through hydroxylation.[2,20,27–29] Oxime acetic acid and nitroso-Gly are subsequently produced *via* dehydration, and the latter can transform into oxime by releasing CO_2. Finally, nitrate is generated from the hydroxylamine formed from oxime acetic acid and oxime in the ozonation through a nitrite intermediate. Some studies also proposed that nitro-Gly/Ser is formed in the ozonation of Gly and serine (Ser), as displayed in Scheme 1(b); nitro-Gly can also be produced from nitroso-Gly, and then it can be ozonized into nitrate *via* nitrite and nitroalkane.[7,24,28] With respect to amines, studies have shown that tertiary amines (TMA/TEA) can degrade into secondary amines (DMA/DEA) through a radical mechanism but with rather high activation free energies.[2,7,31–36] DMA/DEA can be ozonized into hydroxylamine or MA/EA, and the former can transform into nitroalkane through nitrone and nitrosoalkane.[7,8,22,24,29,30,32,37] For primary amines (MA/EA), similar to AAs, oxime and nitrosoalkane can be first generated, and nitrate is obtained from nitroalkane and oxime.[32,33] Overall, it can be found that several pathways for nitrate formation have been proposed. Which one is more likely to occur and which intermediate is more important for AAs and amines, oxime or nitroalkane? Additionally, it is important to uncover which process is the key step to determine the molar yields of nitrate and which characteristic parameter can be used to identify the reactivity of various precursors.

To answer the above questions, in this study, nitrate formation mechanisms from model AAs including Gly and Ser and model amines including MA, EA, BA, DMA, DEA, TMA, and TEA during ozonation were first investigated by quantum chemical computations. To verify the reliability of our proposed mechanisms, additional precursors were chosen and the relationship between the activation free energies ($\Delta G^{\neq}$) of the important steps for all precursors and their experimental nitrate molar yields was studied. Finally, the comparison of nitrate formation from AAs and amines along with some correlation analyses was carried out. The results are expected to expand our understanding of nitrate formation mechanisms and to assist in predicting the nitrate yields of different free AAs and amines.

Table 1 Rate constants (k_{O_3}, $M^{-1}\ s^{-1}$) (at 298 K and 1 atm) and nitrate molar yields for ozonation reactions of AAs and amines obtained from the literature at the O/N ratio of 5 unless specified otherwise

Name	k_{O_3}	Yields (%)	Name	k_{O_3}	Yields (%)
Amino acids			**Primary amines**		
Ser	$1.3 \times 10^{5\ a}$	57.0 ± 0.7	4-Nitrobenzylamine		71.7 ± 0.7
Gly	$2.1 \times 10^{5\ a}$	54.8 ± 0.5	4-Chlorobenzylamine		41.7 ± 1.2
Glu	$2.6 \times 10^{4\ a}$	54.3 ± 0.5	Benzylamine (BA)	$6.3 \times 10^{4\ a}$	33.0 ± 0.6
Ala	$7.6 \times 10^{4\ a}$	52.8 ± 0.5	Ethanolamine		22.0 ± 1.4
Phe	$3.8 \times 10^{5\ a}$	52.3 ± 0.3	Ethylamine (EA)	$9.3 \times 10^{4\ b}$ $2.4 \times 10^{5\ c}$	8.5 ± 1.6
Asp	$4.1 \times 10^{4\ a}$	40.2 ± 1.3	β-Alanine		7.6 ± 1.3
Cys	$3.1 \times 10^{4\ a}$	30.5 ± 1.3	Methylamine (MA)	$<1 \times 10^{5\ a}$	
Secondary amines			**Tertiary amines**		
Dimethylamine (DMA)	$1.2 \times 10^{7\ a}$		Trimethylamine (TMA)	$4.1 \times 10^{6\ a}$ $5.1 \times 10^{6\ c}$	1.5 ± 0.3
Diethylamine (DEA)	$3.9 \times 10^{5\ b}$ $6.2 \times 10^{5\ a}$ $9.1 \times 10^{5\ c}$	6.5 ± 1.3	Triethylamine (TEA)	$2.2 \times 10^{6\ b}$ $2.1 \times 10^{6\ d}$ $4.1 \times 10^{6\ c}$	
N-methyl-benzylamine		19.0 ± 0.8	Tramadol		2.6 ± 0.3
Sarcosine		3.6 ± 0.2	*N*,*N*-Dimethyl-benzylamine		3.1 ± 0.2

[a] Pryor *et al.*, 1984. [b] Lim *et al.*, 2019 ($[O_3]$ ranges from 0 to 300 μM, [initial org-N] = 100 μM, *T*: 4 °C, *t*: 24 h). [c] Muñoz *et al.*, 2000. [d] Furuhama *et al.*, 2018.[26]



Scheme 1 Nitrate formation mechanisms from AAs and amines during ozonation proposed in the literature.

2 Computational methods

All quantum chemical computations were carried out with the GAUSSIAN-09 program package.[38] Since previous studies[7,29,32] demonstrated that it is molecular ozone rather than the HO$^{\bullet}$ radical that plays a major role in nitrate formation during ozonation of amino acids (AAs) and amines, thus, only the ozone molecule was considered in this study. Because no reliable DFT functionals have been reported for specific ozonation reactions of AAs in aqueous solution, three DFT functionals M05,[39] M06,[40] and LC-ωPBE[41] along with the 6-31G(d), 6-311G(d), 6-31+G(d), and 6-311+G(d) basis sets,[42] as well as SMD[43] and CPCM[44] implicit solvent models were used to investigate ozonation reactions with Gly, Ser, and Ala. All structures of the reactants, transition states, and products were fully optimized. Vibrational frequencies were calculated at the same level of theory to characterize the nature of the stationary points. The minimum-energy path (MEP) was obtained using intrinsic reaction coordinate (IRC) calculations to confirm the connection between each transition state and the corresponding reactant and product.[45] To obtain more accurate energies, single point calculations were performed for some important reactions using the present most reliable benchmark CCSD(T) method along with the 6-311+G(d) basis set based on the optimized structures. The activation free energies ($\Delta G^{\neq}$) and reaction free energy changes (ΔG) (at 298 K and 1 atm) for each elementary reaction are defined below.

$$\Delta G^{\neq} = G(\text{transition state}) - G(\text{reactant complex}); \Delta G = \sum G(\text{products}) - \sum G(\text{reactants})$$

Moreover, all the transition states in the reactions involving ozone were treated with open shell DFT methods. In this case, the broken-symmetry approach was used to obtain a stable wave function solution and stability tests were routinely carried out due to the possibility of biradical characteristics for transition structures. To correct spin contamination, the energies of transition states were refined based on Yamaguchi's approximate spin-projection method.[46]

$$E^{\mathrm{AP}} = \alpha E^{\mathrm{BS}} - \beta E^{\mathrm{HS}}, \quad \text{where } \alpha = \frac{\hat{S}^{2\mathrm{HS}}}{\hat{S}^{2\mathrm{HS}} - \hat{S}^{2\mathrm{BS}}}$$

$$\beta = \alpha - 1 = \frac{\hat{S}^{2\mathrm{BS}}}{\hat{S}^{2\mathrm{HS}} - \hat{S}^{2\mathrm{BS}}}$$

where AP represents the approximate spin-projection, and HS and BS represent high-spin and broken-symmetry, respectively.

According to transition state theory (TST),[47,48] the approximate reaction rate constants for some elementary reactions, in which the reactants directly generate products through a concerted pathway, were estimated based on the Eyring–Polanyi equation (eqn (1)):

$$k_{\mathrm{est}} = (k_{\mathrm{B}}T/h)\exp(-\Delta G^{\neq}/RT) \tag{1}$$

where k_{B} is the Boltzmann constant, T is the temperature (unit: K), h is Planck's constant, R is the molar gas constant, and $\Delta G^{\neq}$ (unit: kcal mol^{-1}) is the activation free energy relative to the reactant complexes. In view of the C_{α}^{-} anion form (base) of nitroalkane accounting for a small fraction but featuring a high reactivity in the ozonation reactions, its contribution should be considered. As a result, the estimated apparent rate constants ($k_{\mathrm{obs\text{-}est}}$) are calculated based on eqn (2),

$$k_{\mathrm{obs\text{-}est}} = k_{\mathrm{calc}}(\mathrm{base})f(\mathrm{base}) + k_{\mathrm{calc}}(\mathrm{acid})f(\mathrm{acid}) \tag{2}$$

where f(base) is the fraction of the base form with the value of $f(\mathrm{base}) = 1/(1 + [\mathrm{H}^{+}]/K_{\mathrm{a}})$ while f(acid) is the fraction of the acid form with the value of $f(\mathrm{acid}) = 1 - f(\mathrm{base})$.[49] The details about the estimations of the pK_{a} values of nitroalkanes are discussed in Text S1† and values are shown in Table S1.† In turn, the corresponding estimated apparent activation free energies ($\Delta G^{\neq}_{\mathrm{obs\text{-}est}}$) can be obtained by putting $k_{\mathrm{obs\text{-}est}}$ into eqn (1).

Tables S2–S4† show that the $\Delta G^{\neq}$ values of ozone reacting with Gly, Ser, and Ala calculated at the M05/6-311G(d) level with the CPCM solvent model are 10–11 kcal mol^{-1} with estimated rate constants k_{est} of $\sim 10^{5}$ M^{-1} s^{-1}, which agree well with the experimental rate constants k_{exp} of 7.6×10^{4} to 2.1×10^{5} M^{-1} s^{-1}.[23] This supports the conclusion that the M05 functional has a uniquely broad ability to predict reasonably accurate barrier heights of ozone reactions.[50] Therefore, the nitrate formation from AAs under ozonation was investigated at the M05(CPCM)/6-311G(d) level unless otherwise stated, where the 6-311+G(d) basis set was used for anion systems in the degradation reactions. Regarding the ozonation of amines, based upon our previous work,[32] Table S5† demonstrates that the $\Delta G^{\neq}$ values for tertiary, secondary, and primary amines, *i.e.*, TMA/TEA, DMA/DEA, and MA/EA, calculated at the LC-ωPBE(CPCM)/6-311++G(d,p) level are in the range of 8–10 kcal mol^{-1} with the k_{est} of $\sim 10^{5}$ to 10^{6} M^{-1} s^{-1}, which agree well with the k_{exp} of 10^{4} to 10^{6} M^{-1} s^{-1}.[2,6,7,26,31,51] However, the $\Delta G^{\neq}$ values calculated at the M05(CPCM)/6-311++G(d,p) level are 13–14 kcal mol^{-1} with the k_{est} of $\sim 10^{2}$ to 10^{3} M^{-1} s^{-1}, lower than those of k_{exp}. Notably, in contrast to AAs, the LC-ωPBE functional is more suitable than M05 for the ozonation of amines. Therefore, the nitrate formation from amines under ozonation was investigated at the LC-ωPBE(CPCM)/6-311++G(d,p) level.

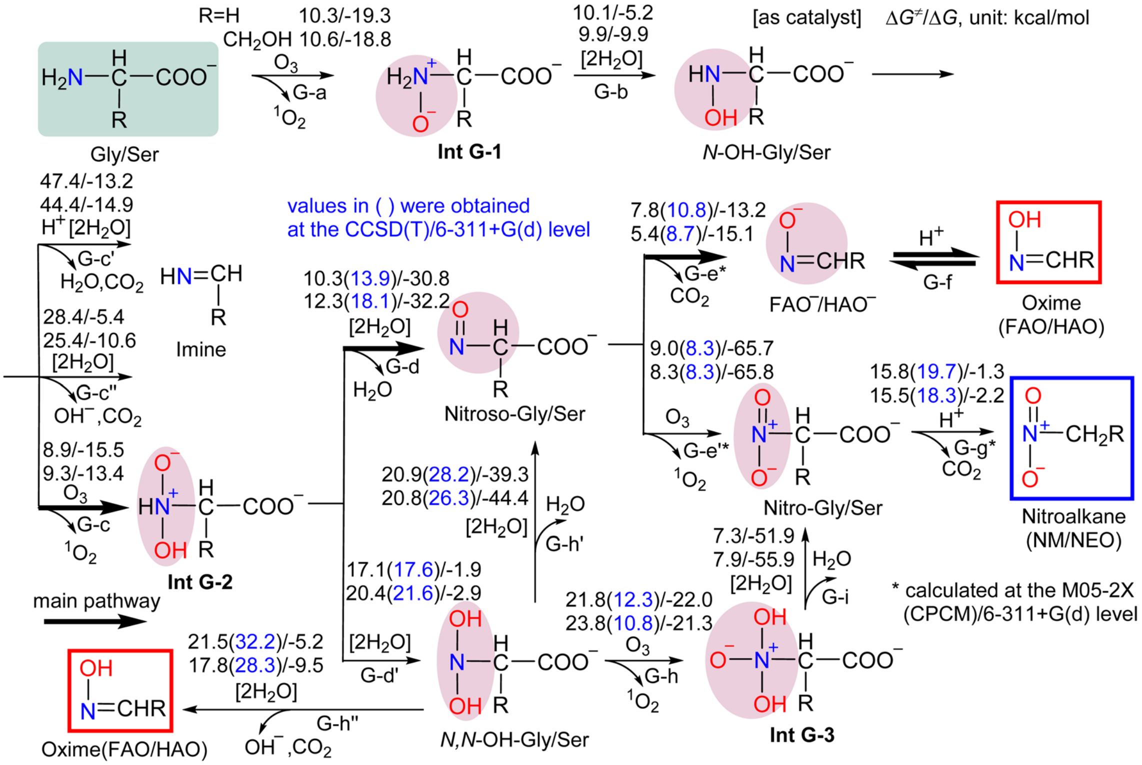
3 Results and discussion

3.1 Nitrate formation from free AAs during ozonation

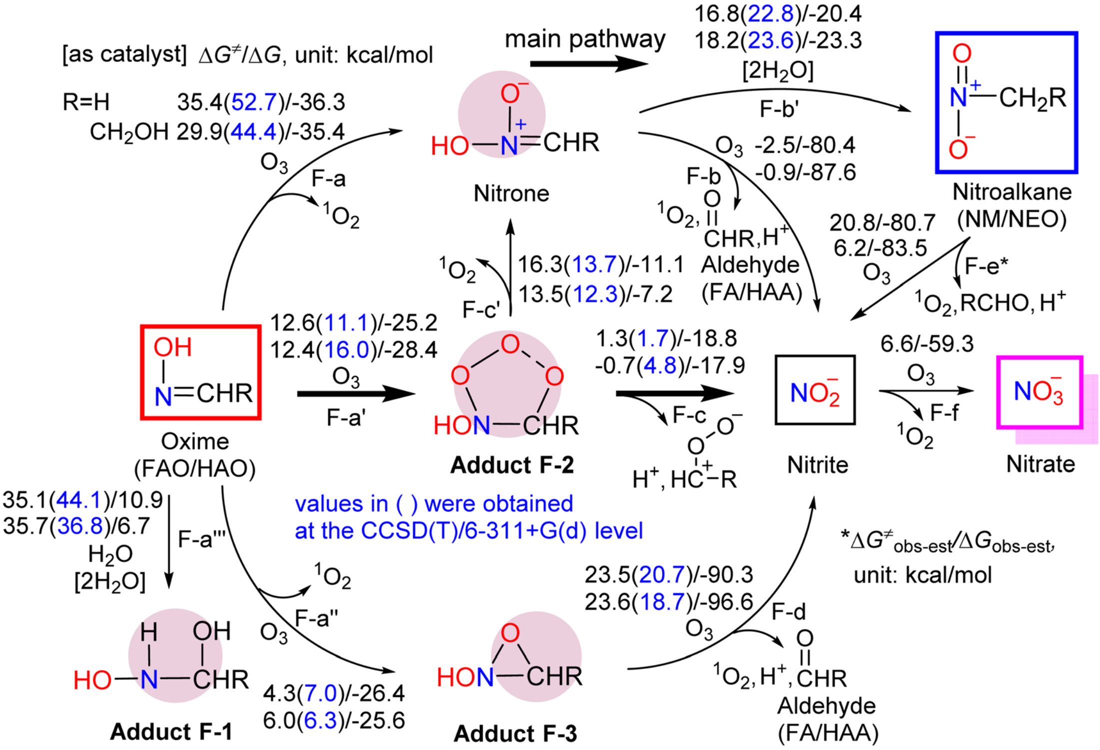
3.1.1 Nitrate formation mechanism from model AAs. Gly and Ser were chosen as model AAs to investigate the nitrate formation mechanism during ozonation. The reaction pathways of oxime formation (formaldehyde oxime (FAO) and 2-hydroxyacetaldehyde oxime (HAO)) from Gly and Ser, and nitrate generation from oxime during ozonation are depicted in Schemes 2 and 3, respectively, and the geometries of their transition states are illustrated in Fig. S1 and S2,† respectively.

As shown in Scheme 2, similar to (m)ethylamine, *N*-ozonation reaction initially occurs to generate *N*-monohydroxyl-AAs (*N*-OH-Gly/Ser) for Gly and Ser *via* steps G-a and G-b with $\Delta G^{\neq}$ values of 10–11 kcal mol^{-1}. Among these steps, the first oxygen-transfer (step G-a) is the rate-limiting step with k_{est} of $\sim 10^{5}$ M^{-1} s^{-1}, in good agreement with k_{exp} of 1.3×10^{5} to 2.1×10^{5} M^{-1} s^{-1}.[23] Similar to *N*-Cl-AAs,[52] *N*-OH-Gly/Ser can follow three reaction pathways including ozonation (step G-c) to form **Int G-2** along with dehydration (step G-c′) and concerted Grob fragmentation (CGF) (step G-c″) to generate imine. Notably, dehydration and CGF reactions for *N*-OH-Gly/Ser possess rather high $\Delta G^{\neq}$ values of 44–47 and 25–28 kcal mol^{-1}, respectively, implying that they are kinetically less feasible relative to those for *N*-Cl-AAs.[52] This is mainly due to the difference of chlorination and ozonation, in which the OCl^{-} anion exists as a base to catalyze the reaction in the chlorination, whereas this kind of base does not exist in the ozonation. However, the ozonation reaction (step G-c) of *N*-OH-Gly/Ser is kinetically more feasible than the above reactions, occurring with a $\Delta G^{\neq}$ value of 9 kcal mol^{-1} and k_{est} of $\sim 10^{6}$ M^{-1} s^{-1}, in line with the k_{exp} of 2.0×10^{4} to 4.6×10^{6} M^{-1} s^{-1} for the ozonation of hydroxylamine.[32,53] Interestingly, the second ozonation has slightly lower $\Delta G^{\neq}$ values than the first one, while the second chlorination has much higher $\Delta G^{\neq}$ values by $\sim$10 kcal mol^{-1} than the first one.[52,54] The above results indicate that the twice-ozonized products are more readily produced compared to the imines easily formed in chlorination. Afterwards, **Int G-2** undergoes competitive dehydration and hydrogen atom transfer (HAT) reactions to yield the respective nitroso-Gly/Ser and *N*,*N*-dihydroxyl-AAs (*N*,*N*-OH-Gly/Ser) through steps G-d and G-d′ with $\Delta G^{\neq}$ values from the CCSD(T) method of 14–18 and 18–22 kcal mol^{-1}, respectively, indicating that the generation of nitroso-Gly/Ser is kinetically more favorable compared to *N*,*N*-OH-Gly/Ser. However, the close $\Delta G^{\neq}$ values imply that they are both likely to be formed.

Then nitroso-Gly/Ser undergoes decarboxylation (step G-e) and ozonation (step G-e′) reactions to produce the



Scheme 2 Oxime (FAO/HAO) formation pathways from Gly and Ser during ozonation.



Scheme 3 Nitrate formation pathways from oxime (FAO/HAO) during ozonation.

FAO^-/HAO^- anion and nitro-Gly/Ser. However, unfortunately, no correct reactant in step G-e′ was obtained in the IRC calculations with the M05 method. As demonstrated in Fig. S3,† the carboxyl group is finally released from the reactant after the observation of the "shoulder". Accordingly, M05-2X, M06-2X, ωB97X-D, BMK, and LC-ωPBE functionals were used to investigate this ozonation reaction (step G-e′) and its subsequent reaction (step G-g) as well as the branching reaction of nitroso-amino acids (step G-e). Table S6† shows that the $\Delta G^{\neq}$ values of all functionals except for the LC-ωPBE functional are very close to each other, indicating the results from these functionals are reasonable, therefore, in Scheme 2, only the $\Delta G^{\neq}$ values of M05-2X were depicted on their behalf. Scheme 2 shows that the $\Delta G^{\neq}$ values from the CCSD(T) method for the FAO$^-$/HAO$^-$ anion and nitro-Gly/Ser formation *via* steps G-e and G-e′ are 9–11 and 8 kcal mol^{-1}, respectively, where the dominant species of the former is the neutral FAO/HAO at pH 7 due to their calculated pK_a of ∼11. Such close $\Delta G^{\neq}$ values imply that both reactions are kinetically feasible. Moreover, nitro-Gly/Ser formation is kinetically more feasible than oxime generation due to the relatively lower activation free energies. However, the $\Delta G^{\neq}$ values of nitroalkane generation from nitro-Gly/Ser (step G-g) are 18–20 kcal mol^{-1}, relatively higher than those of steps G-e and G-e′. This indicates that nitroalkane formation from nitroso-Gly/Ser is less favorable than oxime generation. For *N*,*N*-OH-Gly/Ser, like *N*–OH-Gly/Ser, ozonation (step G-h) has lower $\Delta G^{\neq}$ values than dehydration (step G-h′) and CGF (step G-h″) reactions with the values from CCSD(T) of 11–12, 26–28, and 28–32 kcal mol^{-1} to generate **Int G-3**, nitroso-Gly/Ser, and FAO/HAO, respectively. **Int G-3** can then transform into nitro-Gly/Ser with $\Delta G^{\neq}$ values of 7–8 kcal mol^{-1}. This indicates that nitroso-Gly/Ser and *N*,*N*-OH-Gly/Ser can both produce oxime and nitroalkane. The $\Delta G^{\neq}$ values of the rate-limiting step ($\Delta G^{\neq}_{\text{rls}}$) for oxime formation from nitroso-Gly/Ser and *N*,*N*-OH-Gly/Ser are 9–11 kcal mol^{-1} for step G-e and 28–32 kcal mol^{-1} for step G-h″, respectively, while those for nitroalkane formation from both are 18–20 kcal mol^{-1} for step G-g. However, one more step (step G-h) with a relatively higher $\Delta G^{\neq}$ (11–12 *vs.* 8 kcal mol^{-1}) is needed for *N*,*N*-OH-Gly/Ser compared to nitroso-Gly/Ser. This indicates that the production of both oxime and nitroalkane is more feasible from nitroso-Gly/Ser than from *N*,*N*-OH-Gly/Ser in terms of kinetics because of the lower $\Delta G^{\neq}$ values and less steps. Comparing oxime and nitroalkane formation from Gly/Ser, the rate-limiting reactions are steps G-d and G-g with $\Delta G^{\neq}_{\text{rls}}$ values of 14–18 and 18–20 kcal mol^{-1}, respectively, indicating that obtaining oxime is kinetically more feasible than obtaining nitroalkane. Additionally, it is notable that in most cases $\Delta G^{\neq}$ values calculated from DFT functionals are somewhat lower than those from the CCSD(T) method. This supports the conclusion that many DFT methods generally underestimate barrier heights compared to CCSD(T) calculations.[55,56]

Regarding nitrate formation from oxime, previous mechanisms proposed that oxime undergoes hydrolysis to produce nitrate.[5,33] However, as shown in Scheme 3, the $\Delta G^{\neq}$ values from CCSD(T) and ΔG values for FAO/HAO reacting with water molecules to form **Adduct F-1** (step F-a‴) are 37–44 and 7–11 kcal mol^{-1}, respectively, indicating that these reactions are neither thermodynamically favorable nor kinetically feasible. Instead, three kinds of ozonation reactions of FAO/HAO including the attack of the N atom, the C=N bond, and the C atom in oxime by ozone were found as reported in previous studies.[32,57,59] These reactions lead to the formation of nitrone, **Adduct F-2**, and **Adduct F-3** through steps F-a, F-a′, and F-a″ with $\Delta G^{\neq}$ values from the CCSD(T) method of 44–53, 11–16, and 6–7 kcal mol^{-1}, respectively. The lower $\Delta G^{\neq}$ values in steps F-a′ and F-a″ relative to that in step F-a indicate that the C atom and C=N bond in oxime are preferably attacked by ozone. Then interestingly, nitrone, **Adduct F-2**, and **Adduct F-3** can all transform into nitrite *via* steps F-b, F-c, and F-d with $\Delta G^{\neq}$ values of −2–0, −1–1, and 24 kcal mol^{-1}, respectively, indicating that nitrite is more likely to be produced from nitrone and **Adduct F-2**. This conclusion is in line with previous work where hydroxyacetaldehyde (HAA, CH_2OH–CHO) as another product produced *via* steps F-c and F-d for R = CH_2OH was observed in the ozonation of Ser.[28,58,59] Additionally, **Adduct F-2** can transform into nitrone *via* step F-c′ with $\Delta G^{\neq}$ values from the CCSD(T) method of 12–14 kcal mol^{-1}, and nitrone can transform into nitroalkane (nitromethane (NM)/nitroethanol (NEO)) *via* step F-b′ with the $\Delta G^{\neq}$ value of 23–24 kcal mol^{-1}. Nitroalkane can also be ozonized into nitrite (step F-e) with $\Delta G^{\neq}$ values of 21 and 6 kcal mol^{-1} for R = H and CH_2OH, respectively. Finally, nitrate can be rapidly generated from nitrite *via* step F-f with a $\Delta G^{\neq}$ value of 7 kcal mol^{-1}, and this reaction is thermodynamically favorable with a ΔG value of −59 kcal mol^{-1}. This suggests that nitrite is unstable and can be easily converted into nitrate under ozonation, which is consistent with previous studies.[35,60] Comparing all the reaction pathways from FAO/HAO to nitrate, pathway FAO/HAO → **Adduct F-2** → nitrite → nitrate is the most probable one and the rate-limiting reaction is step F-a′ with $\Delta G_{\text{rls}}{}^{\neq}$ values of 12–13 kcal mol^{-1}. Such low $\Delta G^{\neq}_{\text{rls}}$ indicates that nitrate formation from oxime is kinetically rather favorable, which provides a good explanation for the previous result that nitrate yield of benzaldehyde oxime is up to ∼100%.[22]

Taking all steps of nitrate formation from Gly/Ser under ozonation into account, it can be found that the rate-limiting steps are the dehydration reaction of **Int G-2** (step G-d) and addition reaction of the C=N bond of intermediate FAO/HAO with ozone (step F-a′) with $\Delta G^{\neq}_{\text{rls}}$ values of 10–13 kcal mol^{-1}. Moreover, oxime is the important last intermediate product for nitrate formation rather than hydroxylamine proposed in some studies,[7,27,37] which provides theoretical support to oxime being the important intermediate proposed in the literature.[7,19,27,29] In addition, the most probable pathway for nitroalkane (NM/NEO) formation is AAs → nitroso-AAs → nitro-AAs → nitroalkane with the $\Delta G^{\neq}_{\text{rls}}$ values of step G-g being 18–20 kcal mol^{-1}, while another pathway is AAs → nitroso-AAs → oxime → **Adduct F-2** → nitrone → nitroalkane with the $\Delta G^{\neq}_{\text{rls}}$ values of step F-b′ being 23–24 kcal mol^{-1}. For aldehyde (FA and HAA) formation from oxime, there are two possible pathways, *i.e.*, oxime → **Adduct F-3** → aldehyde and oxime → **Adduct F-2** → nitrone → aldehyde, and their rate-limiting steps are respective steps F-d and F-a′/F-c′ with $\Delta G^{\neq}_{\text{rls}}$ values of 19–21 and 14–16 kcal mol^{-1}, respectively. These data indicate that nitrate is more likely to be the main product, while nitroalkanes and aldehydes are the minor products in the ozonation of AAs. This conclusion agrees well with the experimental results that molar yields of nitrate from Gly and Ser are 55–57% when the O/N ratio is 5, while yields of NM and HAA are 20–26%.[24,28]

3.1.2 Relationship between the nitrate yields of different free AAs and their $\Delta G^{\neq}_{\mathrm{rls}}$. To expand the application of the model, Glu, Ala, Phe, Asp, Cys, and Ile were chosen as additional free AAs. Based upon the above-obtained nitrate formation mechanisms, two possible rate-limiting steps, steps G-d and F-a′, and important branching reactions of **Int G-2** (step G-d′) and nitroso-AAs (steps G-e and G-e′) as well as the initial ozonation of AAs (step G-a) were all investigated for the above AAs. Due to the similar trend of $\Delta G^{\neq}$ values from the DFT and CCSD(T) methods and the relatively large molecule of the additional AAs, the $\Delta G^{\neq}$ values in this part were only calculated with the DFT method, and all the values are listed in Table 2. In our case, 4–5 O_3 molecules are consumed during nitrate formation from AAs, therefore, experimental nitrate molar yields obtained from the literature at the O/N ratio of 5 are given in Table 2 for comparison.

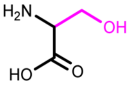
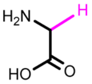
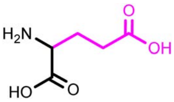
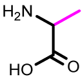
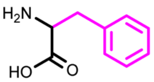
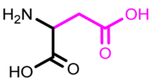
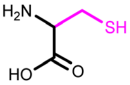
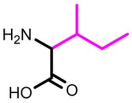
Table 2 indicates that the $\Delta G^{\neq}$ values of step G-a for the eight studied free AAs are 10–12 kcal mol^{-1} with k_{est} of $\sim 10^4$ to 10^5 M^{-1} s^{-1}, which agree well with k_{exp} of 2.6×10^4 to 3.8×10^5 M^{-1} s^{-1}.[23] Notably, all the $\Delta G^{\neq}$ values of step F-a′ are somewhat higher than those of step G-d except for Glu, indicating that step F-a′ is the rate-limiting step for most AAs. Furthermore, the $\Delta G^{\neq}_{\mathrm{rls}}$ values for Ser, Gly, Glu, Ala, and Phe are $\sim$13 kcal mol^{-1}, slightly lower than those (14 and 15 kcal mol^{-1}) for Asp and Cys, respectively. This provides a theoretical explanation for why nitrate yields of these five AAs are higher than those from Asp and Cys (52–57% *vs.* 31–40%).[22] The good relationship between nitrate yields of the above AAs and $\Delta G^{\neq}_{\mathrm{rls}}$ values confirms that the proposed nitrate formation pathway in this study is reasonable and creditable.

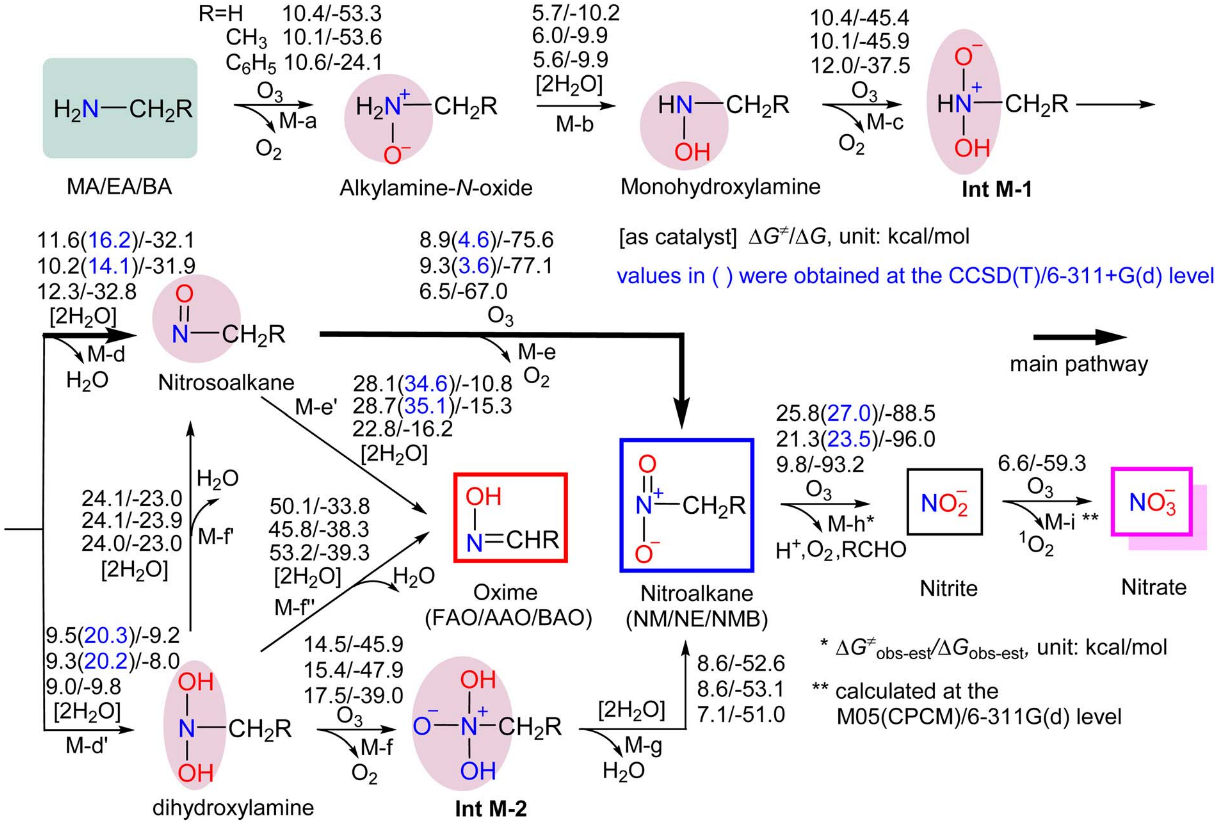
3.2 Nitrate formation from amines during ozonation

3.2.1 Nitrate formation mechanism from model amines. MA, EA, and BA were chosen as model primary amines, and TMA, TEA, DMA, and DEA were chosen as model tertiary and secondary amines. The nitrate formation pathways from primary amines during ozonation are shown in Scheme 4, while those from tertiary and secondary amines are displayed in Scheme 5. The geometries of transition states in nitrate formation from primary amines are demonstrated in Fig. S4.†

As shown in Scheme 4, similar to AAs, *N*-ozonation reaction initially occurs to generate monohydroxylamine for MA/EA/BA through step M-a with $\Delta G^{\neq}$ values of 10–11 kcal mol^{-1} and k_{est} values of $\sim 10^5$ M^{-1} s^{-1}, which is consistent with the values of k_{exp} being approximately 10^4 to 10^5 M^{-1} s^{-1}.[2,6,7,31,51] Similar to the instability of **Int G-1** in Scheme 2, alkylamine-*N*-oxide can rapidly isomerize into monohydroxylamine through HAT with

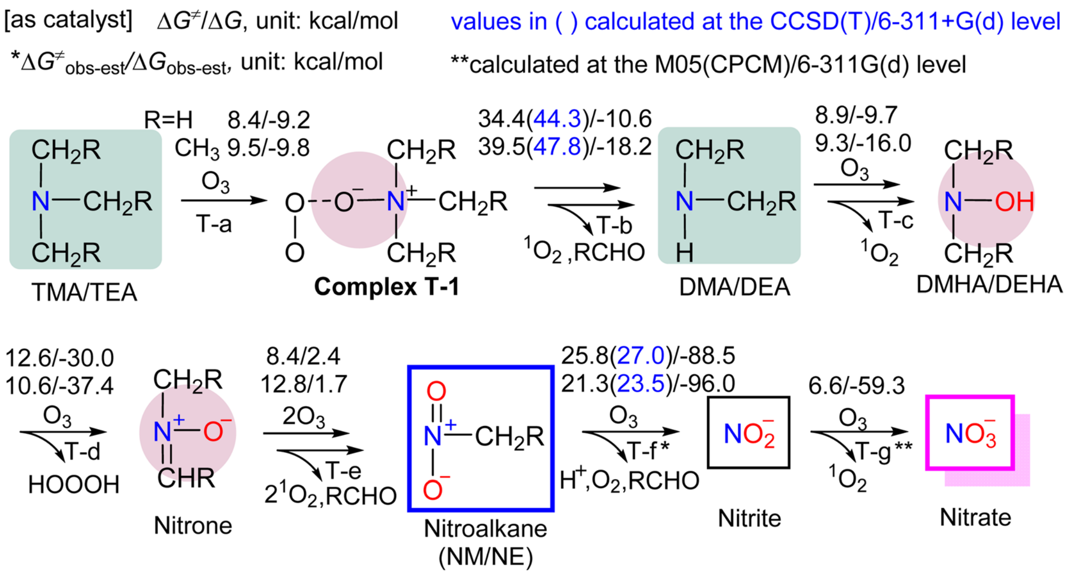
Table 2 $\Delta G^{\neq}$ values (kcal mol^{-1}) of steps G-a, G-d, G-d′, G-e, G-e′, and F-a′ for nitrate formation in the ozonation of AAs and nitrate molar yields obtained from Essaïed *et al.*[22] at the O/N ratio of 5

AAs	Structure formula	Step G-a	Step G-d	Step G-d′	Step G-e	Step G-e′	Step F-a′	Yields (%)
Ser		10.6	12.3	20.4	7.1	7.3	12.4	57.0 ± 0.7
Gly		10.3	10.3	17.1	7.8	9.0	12.6	54.8 ± 0.5
Glu		9.7	12.7	14.9	7.7	7.2	12.4	54.3 ± 0.5
Ala		10.5	12.6	16.4	8.5	9.5	13.4	52.8 ± 0.5
Phe		11.9	12.9	16.2	7.7	6.9	12.9	52.3 ± 0.3
Asp		10.0	11.7	14.7	9.3	6.8	13.6	40.2 ± 1.3
Cys		11.6	13.1	16.3	6.0	9.0	14.7	30.5 ± 1.3
Ile		9.9	10.0	15.9	8.3	7.0	14.5	—



Scheme 4 Nitrate formation pathways from MA/EA/BA during ozonation.

$\Delta G^{\neq}$ values of 6 kcal mol^{-1}. It is notable that the $\Delta G^{\neq}$ values of the above reactions are close to those for Gly and Ser, implying that *N*-ozonation of both AAs and primary amines by ozone is kinetically feasible. Monohydroxylamine can further react with ozone to produce **Int M-1** with $\Delta G^{\neq}$ values of 10–12 kcal mol^{-1} and k_{est} values of $\sim 10^5$ M^{-1} s^{-1}, in line with the k_{exp} value of $\sim 10^5$ M^{-1} s^{-1} for methylhydroxylamine.[7] Then **Int M-1** can undergo dehydration and HAT reactions to produce nitrosoalkane and dihydroxylamine *via* steps M-d and M-d′ with $\Delta G^{\neq}$ values from the CCSD(T) method of 14–16 and 20 kcal mol^{-1},



Scheme 5 Nitrate formation pathways from TMA/TEA and DMA/DEA during ozonation.

respectively. The difference of $\Delta G^{\neq}$ values suggests that dihydroxylamine is less favorable in terms of kinetics to be generated than nitrosoalkane, the same as for their formation from AAs. Then nitrosoalkane can be ozonized into nitroalkane *via* step M-e with $\Delta G^{\neq}$ values from the CCSD(T) method of 4–5 kcal mol^{-1}. Dihydroxylamine transforms into nitroalkane through ozonation and dehydration reactions (step M-f and step M-g) with $\Delta G^{\neq}$ values of 15–18 and 7–9 kcal mol^{-1}, respectively. In addition to nitroalkane, dihydroxylamine can also transform into nitrosoalkane and oxime *via* steps M-f′ and M-f″ with $\Delta G^{\neq}$ values of 24 and 46–53 kcal mol^{-1}, respectively, remarkably higher than those of step M-f. Additionally, nitrosoalkane can transform into oxime *via* keto–enol tautomerism (step M-e′) with $\Delta G^{\neq}$ values from the CCSD (T) method of 35 kcal mol^{-1}. In contrast to AAs, $\Delta G^{\neq}$ values for nitroalkane formation are lower than those for oxime formation from dihydroxylamine and nitrosoalkane. Taking all the reaction pathways from **Int M-1** to nitroalkane formation into account, pathway **Int M-1** → nitrosoalkane → nitroalkane is the most probable one and the rate-limiting reaction is step M-d with $\Delta G^{\neq}$ values of 10–12 kcal mol^{-1}. Afterwards, nitrite forms from nitroalkanes of NM, NE, and NMB under ozonation (step M-h) with $\Delta G^{\neq}_{\text{obs-est}}$ values of 26, 21, and 10 kcal mol^{-1}, respectively, and finally nitrate generates rapidly. In step M-h, as shown in Table S7,† $\Delta G^{\neq}$ values of the neutral species are 30–44 kcal mol^{-1}, while those of their anions are 4–15 kcal mol^{-1}, implying that the C_{α}^{-} is the real reaction site attacked by ozone. Although the fractions of anions at pH 7 are rather tiny due to their calculated pK_a values of 11–15, the anions still make the dominant contributions to $\Delta G_{\text{obs-est}}{}^{\neq}$. As is known, an electron-withdrawing group (EWG) bound to the C_{α} atom facilitates release of the C_{α}^{-} anion, which explains why the nitrate yield of NMB is higher than that of NM (57% *vs.* 28%).[22]

Taking all steps of nitrate formation from model primary amines under ozonation into account, it can be found that the rate-limiting steps are the ozonation of nitroalkane (step M-h) for MA and EA and dehydration of **Int M-1** (step M-d) for BA with $\Delta G^{\neq}_{\text{rls}}$ values of 21–26 and 12 kcal mol^{-1}, respectively. However, the rate-limiting step for nitroalkane formation is step M-d and that for oxime generation is step M-e′ with $\Delta G^{\neq}_{\text{rls}}$ values of 10–12 and 23–29 kcal mol^{-1}, respectively. All of this indicates that nitroalkanes are more likely to be the main products, while nitrate and oxime are the minor products in the ozonation of primary amines, in line with the experimental nitroalkane molar yields of MA, EA, and BA being 67–100%,[7,22,24] and the nitrate yields of EA and BA being 9–33% at the O/N ratio of 5.[22]

As shown in Scheme 5, tertiary amines (TMA/TEA) can be easily ozonized into *N*-oxides with $\Delta G^{\neq}$ values of 8–10 kcal mol^{-1} and the k_{est} of 10^5 to 10^6 M^{-1} s^{-1}, which agree well with the k_{exp} of ~10^6 M^{-1} s^{-1}.[2,6,7,26,31,51] However, they degrade with difficulty into secondary amines (DMA/DEA) with $\Delta G^{\neq}$ values from the CCSD(T) method in step T-b being 44–48 kcal mol^{-1} through a molecular mechanism. This is consistent with the literature reports that these *N*-oxides are the stable products.[32,35] DMA/DEA can then transform into nitroalkanes of NM/NE through DMHA/DEHA and nitrone intermediates with $\Delta G^{\neq}_{\text{rls}}$ values of ~13 kcal mol^{-1}, and subsequently NM and NE can be ozonized into nitrite with $\Delta G^{\neq}_{\text{obs-est}}$ values of 27 and 24 kcal mol^{-1}, respectively, which is the same as in reaction step M-h in Scheme 4. Finally, nitrate is rapidly generated from nitrite during ozonation. Taking all steps of the nitrate formation mechanism from TMA/TEA and DMA/DEA into account, the $\Delta G^{\neq}_{\text{rls}}$ values of TMA/TEA are 44–48 kcal mol^{-1} in step T-b, while those of DMA/DEA are 24–27 kcal mol^{-1} in step T-f, which could explain why the experimental nitrate molar yield of TMA is less than that of DEA (1.5% *vs.* 6.5%) at the O/N ratio of 5.[22]

3.2.2 Relationship between the nitrate yields of different amines and their $\Delta G^{\neq}_{\text{rls}}$. To expand the application of the model, primary amines including α-methylbenzylamine, ethanolamine, and 4-methyl-, 4-chloro-, and 4-nitro-benzylamines, as well as secondary and tertiary amines including *N*-methylbenzylamine, *N*,*N*-dimethylbenzylamine, and sarcosine were chosen. Based upon the above results, the potential rate-limiting steps including steps M-a, M-c, M-d, and M-h for primary amines and steps T-f and T-b for the respective secondary and tertiary amines were investigated using the DFT method. Table S8† shows that step M-h is not the rate-limiting step for all the primary amines and its $\Delta G^{\neq}$ values range from 3 to 29 kcal mol^{-1}. Such a wide range of $\Delta G^{\neq}$ values for step M-h is more likely to be related to the quite different nitrate yields of different primary amines (9–72%). Similar to AAs, 5–7 O_3 molecules are required for nitrate formation from amines, therefore, the $\Delta G^{\neq}$ values of the nitrate yield-controlling steps M-h, T-f, and T-b for the respective primary, secondary, and tertiary amines ($\Delta G^{\neq}_{\text{nycs}}$) along with their nitrate yields at O/N ratio = 5 are listed in Table 3.

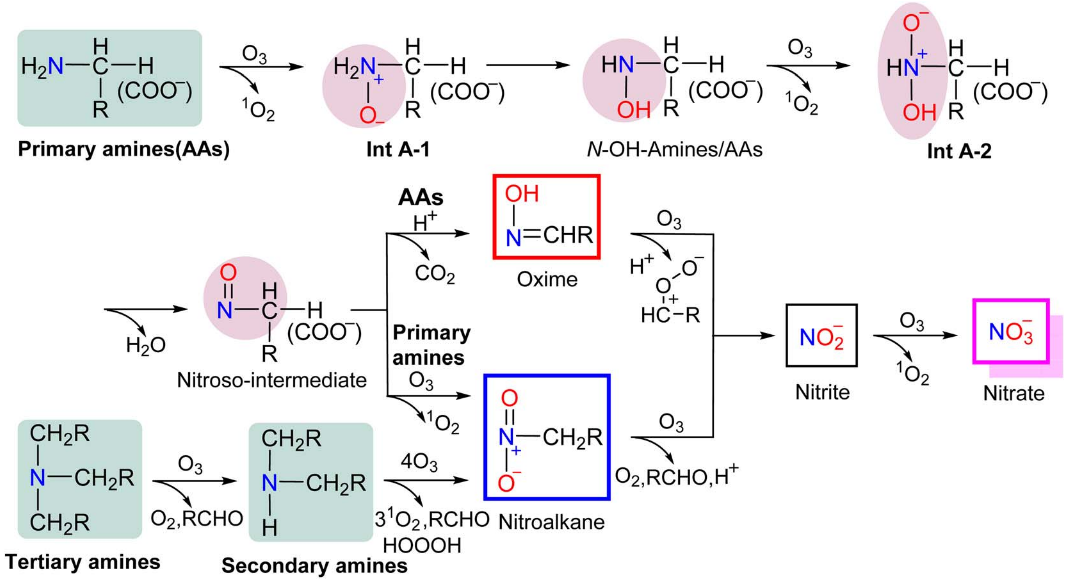
As listed in Table 3, for primary amines, $\Delta G^{\neq}_{\text{nycs}}$ values for MA and EA are 21–26 kcal mol^{-1}, while those for the other 6 primary amines are 3–13 kcal mol^{-1}; obviously, the former is higher than the latter by 8–23 kcal mol^{-1}. This provides a good explanation for why nitrate molar yields from MA and EA are obviously lower than those from the other 6 primary amines (9% *vs.* 19–72%).[22] Regarding secondary amines, the $\Delta G^{\neq}_{\text{nycs}}$ value for *N*-methylbenzylamine is 10 kcal mol^{-1}, while those of DEA and sarcosine are 21–23 kcal mol^{-1}, which agrees well with their nitrate molar yields of 20% and 4–7%, respectively.[22] For tertiary amines, the $\Delta G^{\neq}_{\text{nycs}}$ values for *N*,*N*-dimethylbenzylamine and TMA are 34–35 kcal mol^{-1}, whose nitrate molar yields are 2–3%.[22] The above good relationship between the $\Delta G^{\neq}_{\text{nycs}}$ values of amines and their nitrate yields supports the view that the proposed nitrate formation pathway from amines is believable.

3.3 Comparison of nitrate formation from AAs and amines and correlation analyses

Based upon the above results, Scheme 6 illustrates the main nitrate formation pathways from AAs and amines. It can be found that *N*-ozonation initially occurs for both AAs and primary amines. After two rounds of *N*-ozonation, the nitroso-intermediate is preferred to the *N*,*N*-dihydroxy intermediate in the competitive branching reactions for nitrate formation. In the further reaction of the nitroso-intermediate, due to the decarboxylation occurring relatively more easily than the

Table 3 Calculated $\Delta G^{\neq}$ values of steps M-h, T-f, and T-b for respective primary, secondary, and tertiary amines ($\Delta G^{\neq}_{\text{nycs}}$) and their nitrate molar yields obtained from Essaïed *et al.*[22] at the O/N ratio of 5

Name	$\Delta G^{\neq}_{\text{nycs}}$	Yield (%)	Name	$\Delta G^{\neq}_{\text{nycs}}$	Yield (%)
Primary amines – step M-h			Secondary amines – step T-f		
Methylamine (MA)	25.8	—	*N*-Methylbenzylamine	9.8	19.0 ± 0.8
Ethylamine (EA)	21.3	8.5 ± 1.6	Diethylamine (DEA)	21.3	6.5 ± 1.3
α-Methylbenzylamine	12.6	19.0 ± 0.8	Sarcosine	23.2	3.6 ± 0.2
Ethanolamine	11.7	22.0 ± 1.4	Dimethylamine (DMA)	25.8	—
4-Methylbenzylamine	11.3	23.0 ± 5.4	Tertiary amines – step T-b		
Benzylamine (BA)	9.8	33.0 ± 0.6	*N*,*N*-Dimethylbenzylamine	34.7	3.1 ± 0.2
4-Chlorobenzylamine	9.3	41.7 ± 1.2	Trimethylamine (TMA)	34.4	1.5 ± 0.3
4-Nitrobenzylamine	3.3	71.7 ± 0.7	Triethylamine (TEA)	39.5	—



Scheme 6 Main nitrate formation pathways from AAs and amines during ozonation.

ozonation for the nitroso-intermediate of AAs, oxime and nitroalkane are subsequently generated for AAs and amines, respectively, which are the important last intermediate products for nitrate formation.

Moreover, the ozonation of the above last intermediate products was found to be the nitrate yield-controlling step for AAs and amines. Taking a close look at the mechanism of these reactions, it can be found that ozone attacks the C=N moiety in oxime and the C_α atom in nitroalkane. It is due to the relatively higher reactivity of the C=N moiety in oxime compared to the general C_α atom in nitroalkane in the ozonation reaction that nitrate yield of most AAs is higher than that from general amines. For nitroalkane with an EWG bound to the C_α atom, more C_α^- anions can be released, which are the real reaction sites attacked by ozone, leading to the $\Delta G^{\neq}_{\text{nycs}}$ value being lower, which explains why such nitroalkane has higher nitrate yield.

The quantitative relationship between the $\Delta G^{\neq}_{\text{rls}}$ values for 7 AAs as well as the $\Delta G^{\neq}_{\text{nycs}}$ values for 12 amines and their nitrate yields at the O/N ratio of 5 was explored using the single variate linear regression analysis. The square of the correlation coefficient (R^2) value of all precursors is 0.46 (see Fig. S5(a)†). When separating them back into AAs and amines, as shown in Fig. 1(a), R^2 is 0.89 for 7 AAs, while that for the amines is 0.65 and it increases to 0.85 when tertiary amines and 4-nitrobenzylamine are excluded, as displayed in Fig. 1(b), and the nitro group of the latter may contribute to the nitrate yields. The linear equations of correlations for AAs and amines are listed in eqn (3) and (4), respectively. In addition, considering the important branching reactions likely to play a role in nitrate yields, the relationship between the ratios of the k_{est} of steps G-d/G-d′ along with G-e/G-e′ for 7 AAs and the nitrate yields was first explored. The R^2 of the former is 0.14, lower than that (0.67) of the latter, probably due to the relatively large difference between the $\Delta G^{\neq}$ values in steps G-d and G-d′. Similarly, the large difference in the $\Delta G^{\neq}$ values of steps F-a′ and F-a″ implies that its contribution is even less than that from steps G-d/G-d′. Moreover, as shown in Fig. S5(b),† the R^2 of both $\Delta G^{\neq}_{\text{rls}}$ and k_{est}(G-e)/k_{est}(G-e′) ratio is 0.90. The good relationship not only confirms the validity of the proposed nitrate formation pathway in this study, especially the nitrate yield-controlling steps, but also proves the different reaction mechanisms of the nitrate formation between AAs and amines.

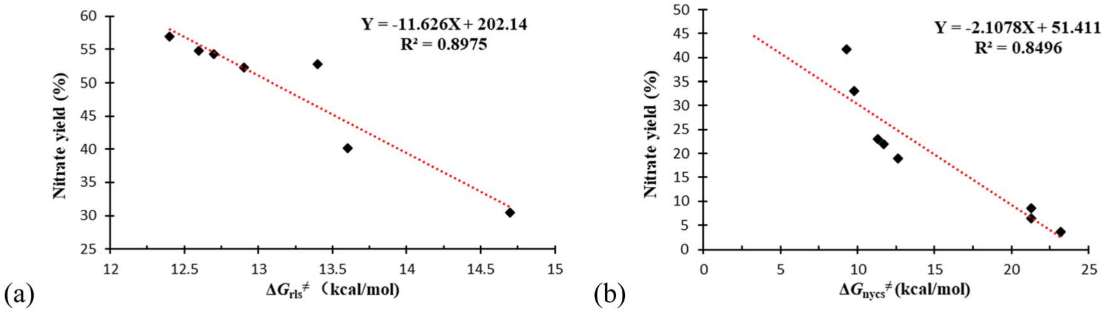


Fig. 1 Relationship between $\Delta G^{\neq}_{\mathrm{rls}}/\Delta G^{\neq}_{\mathrm{nycs}}$ and experimental nitrate yields for (a) 7 AAs and (b) 8 amines.

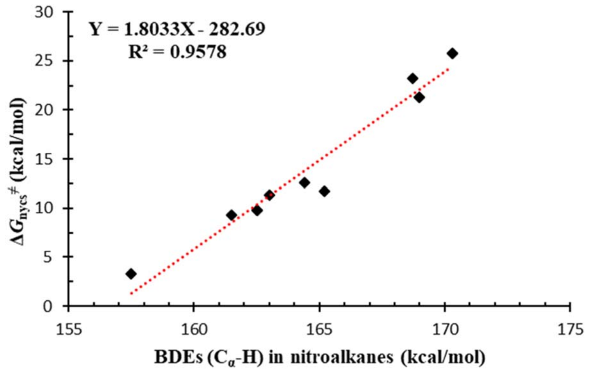


Fig. 2 Correlation between $\Delta G^{\neq}_{\mathrm{nycs}}$ and bond dissociation energies (BDEs) of the C_α–H bond in 9 nitroalkanes formed from amines.

$$\text{Nitrate yields (\%)} = -11.626\Delta G^{\neq}_{\mathrm{rls}} + 202.14 \tag{3}$$

$$\text{Nitrate yields (\%)} = -2.1078\Delta G^{\neq}_{\mathrm{nycs}} + 51.411 \tag{4}$$

Additionally, as shown in Fig. 2, a good correlation between $\Delta G^{\neq}_{\mathrm{nycs}}$ and bond dissociation energies of the C_α–H bond (BDEs(C_α–H)) in 9 nitroalkanes formed from primary and secondary amines was also found with $R^2 = 0.96$ (see data in Table S9†). The linear equation of the above correlation is displayed in eqn (5). This correlation indicates that the BDE(C_α–H) in nitroalkane is a good characteristic parameter to identify the reactivity of the amine, which can be used to predict the nitrate yields of various amines.

$$\Delta G^{\neq}_{\mathrm{nycs}} = 1.8033 \times \mathrm{BDEs}(C_\alpha\text{–H}) - 282.69 \tag{5}$$

4 Conclusion

In this study, nitrate formation mechanisms from model free AAs including Gly and Ser, and model primary, secondary, and tertiary amines including MA, EA, BA, DMA, DEA, TMA, and TEA during ozonation were systematically investigated by quantum chemical computations. The results indicate that oxime and nitroalkane are the important last intermediate products for nitrate formation from AAs and amines, respectively. Moreover, the ozonation of the above important intermediates is the nitrate yield-controlling step and the oxime exhibits higher reactivity in reacting with ozone than the nitroalkane. This leads to the $\Delta G^{\neq}_{\mathrm{rls}}$ values for nitrate formation from most AAs being lower than those from general amines and explains why nitrate yields from AAs are generally higher than those from amines. Furthermore, the experimental results that nitroalkanes with an EWG in the vicinity of the nitrogen atom exhibit higher nitrate yields can be explained by more C_α^- anions being released from these nitroalkanes, which are the real reaction sites attacked by ozone. For tertiary amines, the high $\Delta G^{\neq}_{\mathrm{rls}}$ values for their degradation into secondary amines lead to lower molar yields of nitrate compared to those from most AAs and primary amines (<3% *vs.* 7–60%). To expand the application of the model, an additional 8 kinds of AAs and amines were studied. Based upon the results of all chosen precursors, a good relationship between nitrate molar yields and $\Delta G^{\neq}_{\mathrm{rls}}$ for AAs as well as $\Delta G^{\neq}_{\mathrm{nycs}}$ for amines was found, which further confirms the reliability of the proposed nitrate formation pathway especially the nitrate yield-controlling steps. Moreover, a good correlation between $\Delta G^{\neq}_{\mathrm{nycs}}$ with BDEs(C_α–H) in nitroalkanes formed from amines was also found, which provides a good parameter to evaluate the reactivity and to predict nitrate molar yields from amines. The findings of this work are helpful for further understanding the ozonation reactions of DON and identifying their potential precursors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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