THE JOURNAL OF PHYSICAL CHEMISTRY C

Article

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Plasma-Induced Destruction of Bacterial Cell Wall Components: A Reactive Molecular Dynamics Simulation

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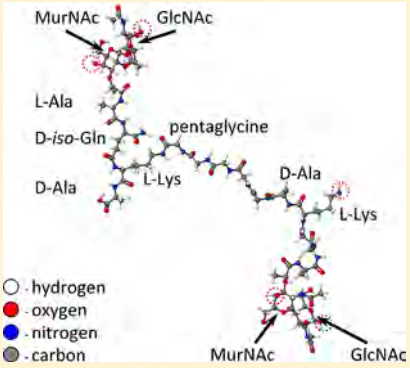
Supporting Information

ABSTRACT: Nonthermal atmospheric pressure plasmas are gaining increasing attention for biomedical applications. However, very little fundamental information on the interaction mechanisms between the plasma species and biological cells is currently available. We investigate the interaction of important plasma species, such as OH, H_2O_2, O, O_3, as well as O_2 and H_2O, with bacterial peptidoglycan by means of reactive molecular dynamics simulations, aiming for a better understanding of plasma disinfection. Our results show that OH, O, O_3, and H_2O_2 can break structurally important bonds of peptidoglycan (i.e., C−O, C−N, or C−C bonds), which consequently leads to the destruction of the bacterial cell wall. The mechanisms behind these breakups are, however, dependent on the impinging plasma species, and this also determines the effectiveness of the cell wall destruction.



1. INTRODUCTION

In recent years, nonthermal or cold atmospheric pressure plasmas (CAPPs) received substantial attention in view of their envisaged biomedical applications,[1−4] including tooth bleaching,[5] sterilization of surfaces,[3,6] treatment of skin diseases,[7] and apoptosis of cancer cells.[8,9] Currently, one of the main applications of CAPPs is the sterilization of surfaces, that is, disinfection from both Gram negative and Gram positive bacteria.[10−13]

There are many conventional methods that are now used for sterilization of medical devices. A widely used method for the inactivation of fungi, bacteria, viruses, and also bacterial spores is an autoclave. However, a disadvantage of the autoclave is that it is heat-based and can, therefore, cause thermal destruction when used for materials such as plastics, rubbers, fiber, and skin. Because of their relatively simple and inexpensive designs (compared with complicated vacuum systems) as well as their nontoxic and nonthermal nature, CAPPs can replace conventional sterilization methods in the near future.[14]

Decontamination of bacteria by CAPPs can be carried out in both dry and humid air as well as in a liquid solution.[15−18] Many studies have revealed that reactive oxygen species, such as O and OH, play a dominant role in inactivation of bacteria, whereas other plasma-generated components (e.g., UV photons, charged particles, electric fields, and heat) have a minor contribution.[13,19−21]

Despite the growing interest in CAPPs for various biomedical applications, gaining control over the processes occurring in the plasma as well as in the contact region of the plasma with the bio-organisms still remains an open problem. Computer simulations can provide fundamental information about processes occurring in the plasma and more importantly at the surface of living cells. Until now, however, very few modeling efforts have been made, especially with respect to the interaction of plasma with living organisms, such as bacteria.[22−24]

Recently, we have investigated the interaction of oxygen species, that is, O, O_2, and O_3, with bacterial peptidoglycan (PG) by means of reactive molecular dynamics (MD) simulations.[23] These species are formed in the plasma, but it is not certain that they can really interact with bacteria, as they might react in the liquid layer surrounding the cells, forming other species, such as OH, H_2O_2, as well as H_2O molecules.[17,25] These H-containing species are also generated in the plasma itself and might remain stable when penetrating the liquid layer surrounding the bacterial cells. It is also known that H_2O_2 is mainly formed from gas-phase recombination of OH radicals.[26] Hence, OH radicals can act as highly reactive species on their own but also as precursors for the formation of H_2O_2 molecules. The chemical reactivity of these reactive oxygen species warrants the effectiveness of plasmas for decontamination and sterilization purposes.[18,27]

Recently, Sakiyama et al. have predicted by numerical modeling that H_2O_2 molecules are among the dominant species in the afterglow region of a surface microdischarge in humid air at atmospheric pressure.[28] Zhang et al. have revealed

Received: December 30, 2012
Revised: February 19, 2013
Published: February 23, 2013

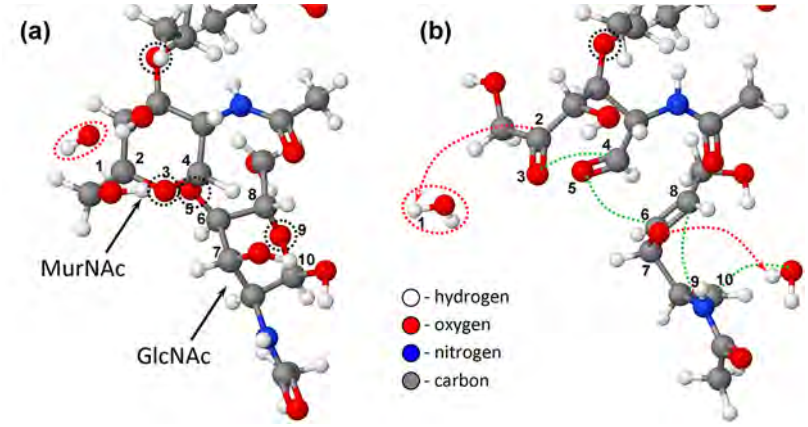


Figure 1. Snapshots from MD simulations, presenting the consecutive breaking of three important ether C−O bonds in the disaccharide (see black dashed circles in MurNAc, GlcNAc, and between them) upon OH radical impact. (a) OH radical (see red dashed circle) first approaches H_1. (b) OH radical abstracts the H_1 atom, which is connected to C_2, forming a water molecule (see red dashed circle). Subsequently, some double bonds are created, which lead to the dissociation of three ether C−O bonds (cf. the bonds between numbered atoms from (a) and (b)). The hydrogen-abstraction reaction and the breaking of bonds are indicated by red dashed arrows and by green dashed lines, respectively.

the presence of, among others, O, OH, H_2O_2, and O_3 in the plasma−liquid system for different operating gases and concluded that the O atoms are the major inactivation agents, while other reactive species (OH, H_2O_2, O_3, etc.) are less important.[13] Hahnel et al.[16] and Bai et al.[17] have suggested that the OH radicals are the key agents responsible for the inactivation of bacteria. Moreover, Xiong et al. have also proposed O and OH as inactivating species because they can easily penetrate into the biofilms and kill the bacteria.[29]

We therefore study the interaction mechanisms of the above-mentioned species (i.e., OH, H_2O_2, O, O_3, as well as O_2 and H_2O) with a bacterial cell wall on the atomic level to elucidate which of these species are indeed important for bacterial cell inactivation. Water molecules are also considered as impinging species, as water vapor is naturally present in atmospheric air, and most bio-organisms, including bacteria, are commonly coated by an aqueous layer surrounding them. Consequently, studying the interaction mechanisms of water molecules with the surface of bacterium (e.g., with the PG) is also of significant interest.

More specifically, we consider the interaction of the above-mentioned species with PG of the Gram positive bacterium *Staphylococcus aureus*. PG is the outer layer of the cell wall of Gram positive bacteria and can therefore directly interact with plasma species. Moreover, it serves as a protective barrier, and its destruction can therefore lead to structural and chemical damage of the entire bacterium.

PG is composed of repeating units consisting of a disaccharide (i.e., *N*-acetylglucosamine and *N*-acetylmuramic acid (GlcNAc-MurNAc)), a stem (i.e., L-alanine-D-*iso*-glutamine-L-lysine-D-alanine (L-Ala_1-D-*iso*-Gln_2-L-Lys_3-D-Ala_4)), and a bridge (i.e., pentaglycine (Gly_1-Gly_2-Gly_3-Gly_4-Gly_5) interpeptide). (See the Supporting Information.) The bridge connects one PG chain to the D-Ala_4 of a neighboring chain. The most important part of the PG (i.e., the part in which most bond-breaking events take place) is shown in Figure 1 below.

Important bonds in the PG structure are C−C, C−N, and C−O bonds. The latter are of importance only in the disaccharides, that is, ether bonds in MurNAc, GlcNAc, in between them and in the residue of MurNAc (see Figure 1 below); dissociation of these bonds can lead to the destruction of PG.[30] Breaking of other C−O bonds in the disaccharides as well as in other parts of the PG is unlikely, as most of these other C−O bonds are double bonds.

2. COMPUTATIONAL DETAILS

Our MD simulations are based on the ReaxFF reactive force field[31] using the C/H/O/N glycine/water parameters developed by Rahaman et al.[32] The PG structure is placed in a box with dimensions ~75 Å × 88 Å × 51 Å without periodic boundary conditions. Before initiating the particle impacts, the PG structure is equilibrated at room temperature (i.e., 300 K). To obtain statistically valid results for bond-breaking processes and to study all possible damaging mechanisms of PG, we perform 50 runs for each impinging species. At the beginning of each run, 10 incident particles of a single species are randomly positioned at a distance of at least 10 Å around the PG structure and from each other to prevent initial interactions between the impinging particles and the PG structure. The velocity directions of incident plasma species are chosen randomly, and their initial energy corresponds to room temperature. The MD time step is set to 0.1 fs. Each run is followed during 300 ps; we carefully checked that this is sufficiently long to obtain a chemically destructed PG structure, at least if a critical bond in the structure is broken. (See later.) A more detailed account of our simulation methodology can be found elsewhere.[23]

3. RESULTS AND DISCUSSION

Our investigations on the H_2O and O_2 impacts reveal that no bond-breaking events occur in important C−O, C−C, and C−N bonds in PG. These molecules are found to assemble around the PG, having weak attractive nonbonded interactions with the structure (i.e., hydrogen bridge formation). Therefore, we do not consider H_2O and O_2 molecules in our further investigations.

The impacts of OH, O, O_3, and H_2O_2 species do result in important bond breakings, but the mechanisms induced by these species appear to be completely different. Indeed, the breakup mechanism induced by OH, O, and O_3 impacts consists of the dissociation of structurally important bonds (i.e., C−O, C−C, and C−N bonds) in the PG, initiated by a hydrogen-abstraction step by the impinging species. In most cases, the abstraction of hydrogen leads to a cascade of C−O

dx.doi.org/10.1021/jp3128516 | J. Phys. Chem. C 2013, 117, 5993−5998

bond cleavage events, that is, the dissociation of three important ether bonds in disaccharide: one in MurNAc, one in GlcNAc, and one between them. A visual representation of this mechanism for OH impacts is shown in Figure 1. Similar results were presented in ref 23 for the O and O_3 species.

The interaction mechanisms of H_2O_2 molecules with PG differ from the mechanisms of the other investigated plasma species (i.e., OH, O_3, and O). The main difference is that H_2O_2 molecules first dissociatively react with each other, forming HO_2 radicals and water molecules. Subsequently, these HO_2 radicals react with the PG structure. Hence, in all cases, the interaction mechanism is initiated by hydrogen-abstraction, but in the case of H_2O_2, the hydrogen is abstracted from the HO_2 radicals by the PG (mostly by an O atom of the PG structure, see below), whereas in the case of the other plasma species investigated (i.e., OH, O_3, and O), the impinging species abstract a hydrogen from the PG.

As in the case of the other impacts, most of the bond-breaking processes due to H_2O_2 molecules occur in the disaccharide parts of the PG (i.e., dissociation of the important ether C−O bonds; see Table 1 below). Various C−O bond breaking mechanisms are observed in the case of H_2O_2 impacts. The most frequently observed mechanism is illustrated in Figure 2. For clarity, relevant atoms are numbered, and the O and H atoms of the impinging H_2O_2 molecules are colored purple and light blue, respectively.

Table 1. Fraction of Important Bond Dissociations (i.e., C−N, C−O, and C−C Bonds) and Associated Standard Deviations upon Impact of O, O_3, OH, and H_2O_2[a]

incident plasma species	C−N bond breaking events (%)	ether C−O bond breaking events (%)	C−C bond breaking events (%)
O atoms	26 ± 6	78 ± 6	38 ± 7
O_3 molecules	8 ± 4	56 ± 7	26 ± 6
OH radicals	8 ± 4	54 ± 7	14 ± 5
H_2O_2 molecules	0	44 ± 7	12 ± 5

[a]Note that the values are calculated from 50 independent simulations for each incident species.

In a first step, H_2O_2 molecules assemble around the PG as a result of weak attractive nonbonded interactions with the PG structure. (See Figure 2a.) When three H_2O_2 molecules are in close vicinity of each other, they react with each other, dissociating into HO_2 radicals and water molecules in a concerted process (see Figure 2b: red dashed arrows and green dashed line). The energy barrier calculated with nudged elastic band method[33] is ∼0.45 eV, which is low enough for the reaction to occur. Subsequently, one of the HO_2 radicals reacts with the OH residue of GlcNAc. (See Figure 2c.) This OH residue abstracts the hydrogen from the HO_2 radical, forming water (see O_1 with bonded H atoms) and an oxygen molecule. As a consequence, this leads to the simultaneous dissociation of C_2−O_1, C_3−O_4, and C_5−O_6 bonds, the formation of double C_2−C_3 and C_5−O_4 bonds, and the abstraction of a hydrogen connected to O_7 by O_6. (See Figure 2c.) Our simulations predict that the dissociation of other ether bonds is also initiated by hydrogen-abstraction from HO_2 radicals.

Our investigations on H_2O_2 molecules interacting with PG reveal that fewer consecutive dissociations of three important ether bonds take place, in comparison with O_3, OH, and O impacts. (See Figure 1b for the OH impacts.) This can be attributed to the different bond-breaking mechanisms occurring in the case of H_2O_2 (see previous). As a result, the H_2O_2 molecules are slightly less effective in breaking C−O bonds than the other plasma species, as can be deduced from Table 1.

Similarly, they are also less effective in breaking C−N and C−C bonds, as is evident from Table 1. Indeed, dissociation of C−N bonds upon impact by H_2O_2 molecules is not observed at all in our simulations. The O, O_3, and OH species can break C−N bonds, and this is again invariably initiated by the hydrogen-abstraction reaction. The cleavage of C−N bonds is found to occur most often in the alanine parts rather than in other parts of the PG structure.

The bond-breaking mechanisms of C−C bonds are found to be similar for the different plasma species investigated; that is, the dissociation of C−C bonds occurs only in the disaccharides, as part of a consecutive mechanism initiated by the breaking of ether bonds. Indeed, after the homolytic dissociation of C−O bonds, some oxygen atoms form a double bond with a neighboring C atom, and this can lead to the breaking of C−C bonds. Thus, the OH, O_3, O, and H_2O_2 species are only capable of breaking C−C bonds indirectly.

Finally, it is clear from Table 1 that dissociation of the important ether C−O bonds in the disaccharides is observed much more frequently than the dissociation of C−N or C−C bonds in the PG.

To further quantify the bond cleavage processes, we have calculated the average times needed to break the important bonds (i.e., C−O, C−C, and C−N bonds) in the PG. The average times required to dissociate the important bonds are shown in Table 2 for the case of O atom impact. Here we show the average times needed only for breaking of maximum two bonds.

Note that in our simulations, up to seven C−O bond breaking events are observed in one run (see consecutive breaking mechanism of C−O bonds, illustrated in Figure 1 above), but we do not show this in the Table, as we are mainly interested in the initiation of the dissociation of important bonds in the PG. However, more detailed tables for the average times of all bond-breaking events, for all impacting species, can be found in the Supporting Information.

It is clear from Table 2 that the bond-breaking events do not occur immediately after the start of the simulation. Indeed, in most cases, it takes several tens to 100 ps before a bond is broken. Once a C−O bond is broken, it typically takes only a short time before the next C−O bond is broken. Indeed, in the case of O atom impacts, in 71% of the cases the second C−O bond is broken within 2 ps, and in 50% of the cases, the second bond breaking occurs even immediately (i.e., within the same simulation frame, 0.6 ps), and this corresponds to the consecutive breaking of ether C−O bonds (see above). In the case of the other plasma species (i.e., OH, O_3 and H_2O_2), the percentages of the short time differences (i.e., within 2 ps after the first bond breaking) are 43, 58, and 42%, respectively, whereas the percentages of immediately occurring bond breaking (i.e., consecutive breaking of ether C−O bonds) amount to 29, 42, and 42% for OH, O_3, and H_2O_2 impacts, respectively. These results again show that most of the ether C−O bond cleavage processes correspond to the consecutive breaking of three ether bonds.

The presented results for the average times needed for breaking of important bonds again show that the chosen simulation time (i.e., 300 ps) is long enough to observe the bond-dissociation processes in the PG structure.

dx.doi.org/10.1021/jp3128516 | J. Phys. Chem. C 2013, 117, 5993−5998

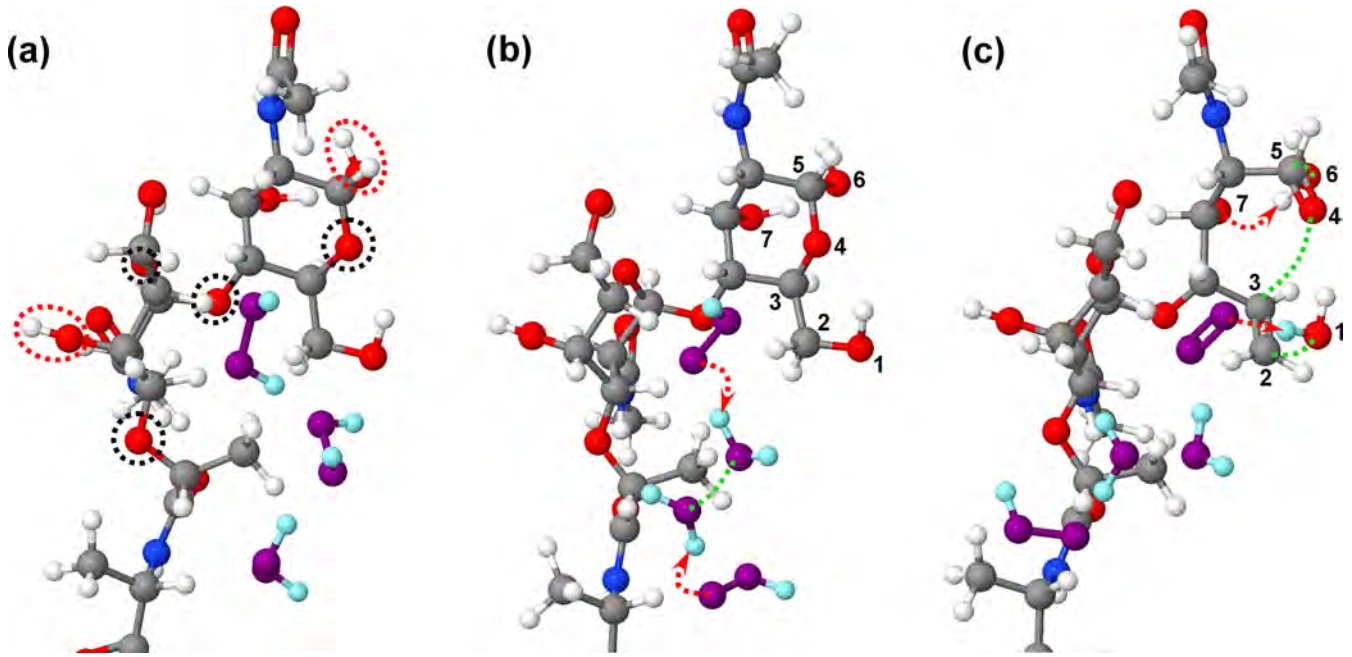


Figure 2. Snapshots from MD simulations, showing the breaking mechanism of the important ether C_3-O_4 bond in GlcNAc upon impact of H_2O_2 molecules. Atoms positioned in the periodically repeating parts of the PG are shown in panel a by red dashed circles, and the ether bonds are indicated by black dashed circles, respectively. H_2O_2 molecules, with the corresponding O and H atoms, are indicated in purple and light-blue colors, respectively. The hydrogen-abstraction reaction and the breaking of bonds are indicated in panels b and c by red dashed arrows and by green dashed lines, respectively. The color legend is identical to Figure 1.

Table 2. Average Characteristic Times Needed to Break the Important Bonds (i.e., C−O, C−C, and C−N bonds) and Associated Standard Deviations, Upon Impact of O Atoms[a]

bonds	bond broken	number of events	average time (ps)
C−O	first	39	53 ± 9
	second	34	63 ± 9
C−C	first	19	98 ± 18
	second	3	89 ± 20
C−N	first	12	85 ± 25
	second	1	37 ± 0

[a]Note that the values are calculated from 50 independent simulations and each simulation time takes 300 ps.

4. CONCLUSIONS

We investigated the interaction of various important plasma species, that is, O, OH, O_2, O_3, H_2O, and H_2O_2 molecules, with bacterial PG on the atomic level by means of reactive MD simulations. We found that O_2 and H_2O molecules cannot structurally damage the PG structure and interact with PG through hydrogen bridge formation. The other plasma species, that is, O, O_3, OH, and H_2O_2, are found to break structurally important bonds of PG (i.e., C−O, C−C, or C−N bonds), which subsequently leads to the destruction of the bacterial cell wall. Our calculations reveal that O_3, OH, and especially O atoms are more effective in bond cleavage than H_2O_2 molecules, in agreement with many experiments demonstrating the crucial role of reactive oxygen species (such as O and OH) in bacteria inactivation.[13,15−21]

Furthermore, the ether C−O bonds in disaccharides are found to break up more easily, followed by the C−C bonds and C−N bonds in the PG. In the case of H_2O_2 molecules, no C−N bond-breaking events are observed, indicating again that the H_2O_2 molecules are somewhat less effective in bacterial cell wall destruction. However, in contrast with the highly reactive O and OH radicals, H_2O_2 (and O_3) molecules are stable species in an aqueous environment and are thus more likely to interact directly with the PG. Hence, we expect that the H_2O_2 molecules are also very important for bacteria deactivation.

The mechanisms of the important bond-breaking processes in the PG were studied in detail. It was found that in all bond cleavage events the dissociation of these bonds is initiated by hydrogen-abstraction. However, a clear difference is observed in the mechanisms upon impact of H_2O_2 molecules on one hand and OH, O, and O_3 species on the other hand. Indeed, in the latter case a H atom is abstracted from the PG by the plasma species (OH, O, or O_3), whereas in the case of H_2O_2 impacts, the H_2O_2 molecules first react with each other, forming HO_2 radicals, from which a H atom is abstracted by an O atom in the PG structure. Abstraction of a H atom from the HO_2 radicals can then cause the dissociation of the important bonds in the PG (i.e., cell wall damage). This corresponds well with experimental observations that hydroperoxyl (HO_2) radicals are strong bactericidal oxidants and can cause the inactivation of the bacteria in an aqueous environment.[12,34]

The average times needed for breaking important bonds are typically on the order of several tens to 100 ps. This shows that the chosen simulation time (i.e., 300 ps) is long enough to observe the bond dissociation processes in the PG. Moreover, once a first C−O bond is broken, it typically takes <2 ps before a second C−O bond is broken (i.e., at least in ∼50% of the cases), which illustrates that the ether C−O bond cleavage process typically proceeds through the consecutive breaking of three ether bonds.

These investigations are highly important for gaining fundamental insight into the mechanisms of plasma species interacting with bacteria and in plasma disinfection in general.

dx.doi.org/10.1021/jp3128516 | J. Phys. Chem. C 2013, 117, 5993−5998

■ ASSOCIATED CONTENT

Supporting Information

Schematic illustration of the *Staphylococcus aureus* peptidoglycan (PG) structure. Atoms positioned in the periodically repeating parts of the PG are indicated by red dashed circles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by FWO-Flanders. This work was carried out in part using the Turing HPC infrastructure at the CalcUA core facility of the Universiteit Antwerpen, a division of the Flemish Supercomputer Center VSC, funded by the Hercules Foundation, the Flemish Government (department EWI), and the Universiteit Antwerpen.

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