Influence of plasma oxidation on proteins: modeling at the molecular level

M. Yusupov[*,1,2,3], M. Ghasemitarei[4], S. Mirzaev[2], U. Khalilov[1,2,3]

[1] National Research University TIIAME, 100000, Tashkent, Uzbekistan
[2] Arifov Institute of Ion-Plasma and Laser Technologies, Academy of Sciences of Republic of Uzbekistan, Durmon yuli str., 33, 100125, Tashkent, Uzbekistan
[3] University of Antwerp, Universiteits plein 1, 2610, Antwerp, Belgium
[4] Sharif University of Technology, Tehran 14588-89694, Iran

Received 20.01.2022

* Corresponding author: e-mail maksudbek.yusupov@uantwerpen.be, Phone: + 998 95 043 92 77

В последнее время использование холодной атмосферной плазмы для лечения рака стало одной из наиболее интересных тем применения плазмы. Несмотря на многообещающие результаты большого количества исследований, основные механизмы воздействия плазмы на раковые клетки все еще не ясны и требуют более тщательных фундаментальных исследований. В этой работе представлен обзор наших последних вычислительных исследований, проведенных для изучения механизмов взаимодействия активных форм кислорода и азота, генерируемых холодной атмосферной плазмой, со специфическими белками, имеющими отношение к раку (лечению). В частности, основное внимание уделено влиянию этих частиц плазмы на проницаемость аквапорина и транспортера цистин/глутамата xCT, используемых в качестве модельных систем для интегральных мембранных белков. Результаты моделирования помогают лучше понять основные механизмы заметного роста плазма-индуцированных реактивных частиц, наблюдаемых в раковых клетках по сравнению с нормальными клетками, тем самым улучшая общее понимание роли интегральных мембранных белков в селективной противоопухолевой способности холодной атмосферной плазмы.

Ключевые слова: холодная атмосферная плазма, плазменное лечение рака, реактивные формы кислорода и азота, интегральные мембранные белки, молекулярная динамика.

Recently, the use of cold atmospheric plasmas for cancer therapy has become one of the most exciting topics of plasma application. Despite promising results from a large number of studies, the underlying mechanisms by which plasma acts on cancer cells are still elusive and require more thorough fundamental investigations. In this account, an overview is provided on our latest computational studies performed to investigate the interaction mechanisms of reactive oxygen and nitrogen species generated by cold atmospheric plasma with specific proteins relevant for cancer (treatment). In particular, the main attention is paid on the effect of these plasma species on the permeability of aquaporin and cystine/glutamate transporter xCT, used as model systems for integral membrane proteins. The simulation results help to gain insight in the underlying mechanisms of the noticeable rise of plasma-induced reactive species observed in cancer cells compared to normal cells, thereby improving overall understanding on the role of integral membrane proteins in the selective anticancer capacity of cold atmospheric plasma.

Keywords: cold atmospheric plasma, plasma cancer treatment, reactive oxygen and nitrogen species, integral membrane proteins, computer simulations, molecular dynamics.

DOI: https//doi.org/10.52304/.v24i1.302

I. Introduction

In recent years, the application of non-thermal atmospheric pressure plasmas operating at near ambient temperature (or simply cold atmospheric plasmas) in cancer treatment is attracting increasing interest and is becoming one of the main topical areas of plasma research [1]. Experiments already evidenced that cold atmospheric plasma may selectively kill cancer cells, leaving homologous normal cells less damaged [2-5]. This and other features of cold atmospheric plasma, such as causing no pain in patients, no thermal and electrical damage, and low cost [6, 7], might give an advantage to cold atmospheric plasma over traditional anticancer therapies.

Cold atmospheric plasma generates reactive oxygen species (ROS) and reactive nitrogen species (RNS). According to the literature, the most relevant ROS generated by cold atmospheric plasma include atomic oxygen (O), singlet delta oxygen ($^1O_2$), superoxide (O_2^-•), ozone (O_3), hydroxyl (•OH) and hydroperoxyl (HO_2•) radicals, and hydrogen peroxide (H_2O_2), whereas the most important plasma-generated RNS are nitric oxide (•NO), nitrogen dioxide (•NO_2), and peroxynitrite ($ONOO^-$) [8, 9]. Some of these reactive oxygen and nitrogen species (RONS), e.g., hydroxyl radical, may have strong oxidizing effects that induce modifications in lipids and proteins, leading to significant alterations in cells. Other RONS, such as nitric oxide, can act as the regulator of the cellular function, thereby influencing the proliferation of cells or immune responses [10, 11].

It is generally believed that the selective anticancer capacity of cold atmospheric plasma is linked to the higher levels of RONS that are generated in cancer cells, while normal cells experience a relatively modest increase (if any) in RONS levels [4, 12-14]. The plasma-induced RONS diffuse across the cell membrane, causing nitro-oxidative stress in the cell, thereby affecting the intracellular signalling pathways, and eventually leading to cell death [15, 16]. However, the underlying mechanisms of the distinct increase of intracellular RONS are still not fully understood, although several explanations have already been proposed in the literatures [17-19].

In this paper, we will provide an overview of our latest computational studies performed using integral membrane proteins [20-23]. These proteins might play an important role in the selective rise of intracellular RONS observed in cancer cells (see Fig. 1 below and explanations therein). Hence, obtained results might give possible explanations to the selective action of cold atmospheric plasma on cancer cells.

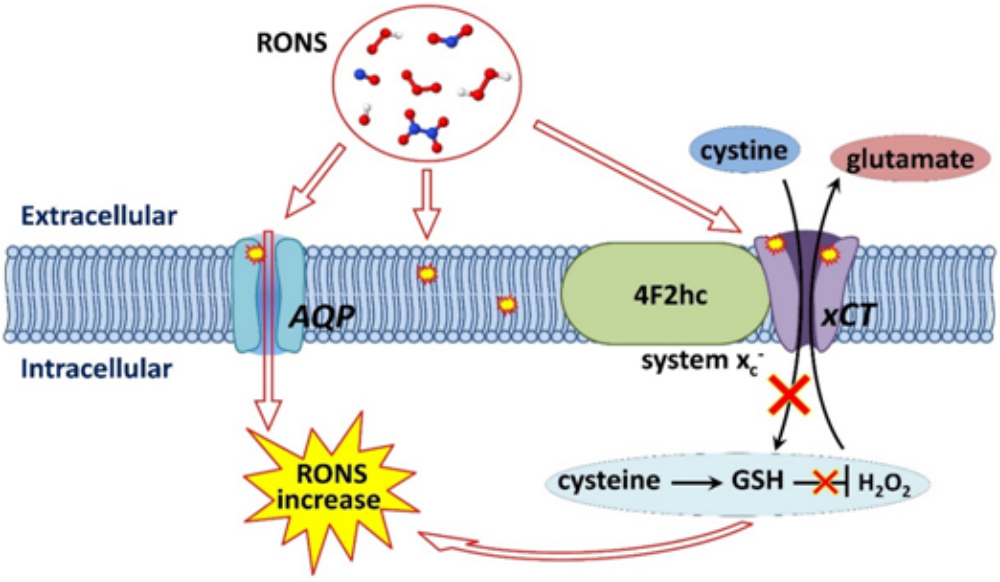


Figure 1. Schematic illustration of the integral membrane proteins aquaporin (AQP) (left) and xCT antiporter (right). Plasma-generated RONS (e.g., H_2O_2) can diffuse across AQP with and without modifying/oxidizing the extracellular part of AQP (yellow star), thereby increasing the intracellular RONS level. These species may also interact with xCT, oxidizing its extracellular part (yellow stars), thereby affecting the xCT function (by e.g., suppressing the cystine permeation), which might also contribute to increasing RONS levels in the cell.

©Academy of Sciences of Republic of Uzbekistan, 2022

II. Simulation setup

All details of the simulation setup are provided in [20-23]. Briefly, we applied atomistic simulations, employing different simulation techniques including molecular dynamics, to study (a) the permeation of RONS through aquaporin (AQP) as well as (b) the transport process of cystine across xCT. As plasma-generated RONS we used H_2O_2 molecule, •OH, •NO and •NO_2 radicals that are in ground states and their spin-states are not taken into account in the simulations.

Both structures (AQP and xCT) were used as model systems for integral membrane proteins in this study (see Fig. 1).We also examined the effect of plasma-induced oxidation (in these model systems) on the transport of the above-mentioned permeants (RONS and cystine). For calculation of the free energy profiles associated with the transport of permeants across model systems, we used the umbrella sampling (US) simulations.

III. Results and discussion

AQPs are trans-membrane proteins that conduct not only water molecules across the cell membrane but also other solutes, such as RONS produced, e.g. by cold atmospheric plasma. In [20] we studied the permeation of H_2O_2 across native AQP1 (one of the members of the AQP family) and lipid membrane, which showed that the delivery of H_2O_2 into the cell interior should be through AQP [20]. However, the overall nature of RONS transport through AQP was still obscure. Therefore, in our next study we extended this research, including more RONS [21].

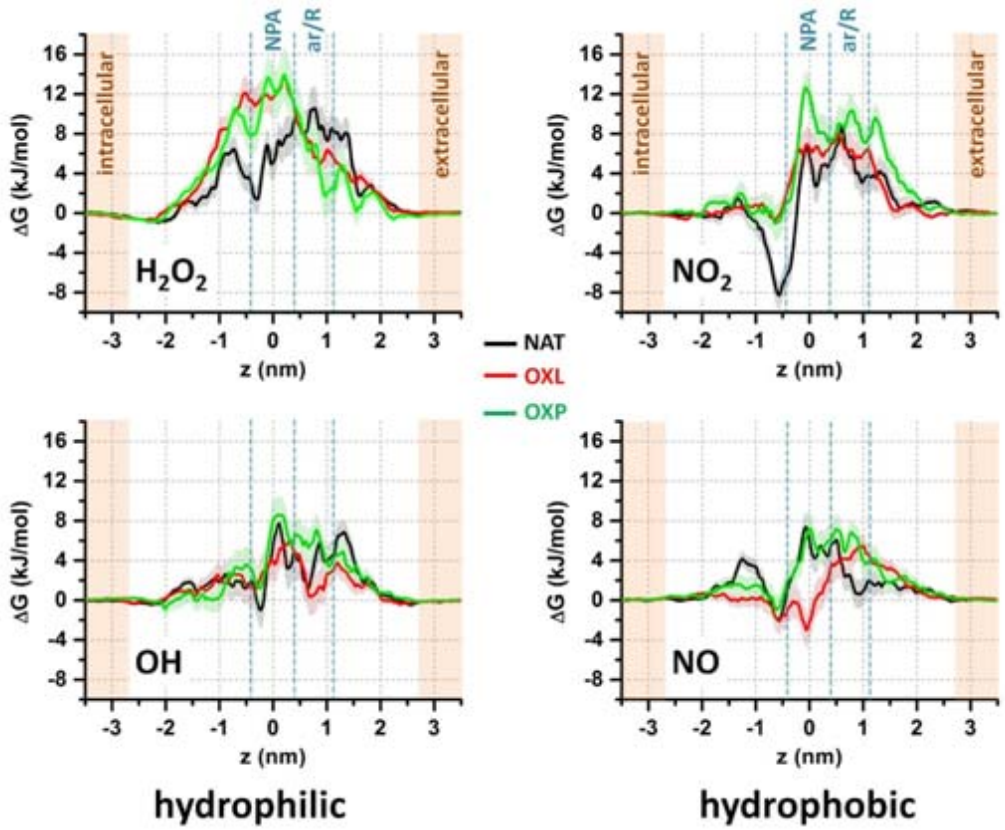


Figure 2. Free energy profiles of the hydrophilic (H_2O_2 and •OH) and hydrophobic (•NO_2 and •NO) RONS across AQP1 pores of the native (NAT, black curve) and oxidized (OXL - oxidized lipids and OXP - oxidized protein, red and green curves, respectively) model systems. The intracellular and extracellular aqueous phases are shown in light blue color. The NPA (asparagine-proline-alanine) and ar/R (aromatic-arginine) regions are indicated by the brown dashed lines. For more details about OXL, OXP, NPA and ar/R, see [21].

©Academy of Sciences of Republic of Uzbekistan, 2022

Specifically, applying molecular dynamics simulations we investigated the permeation processes of both hydrophilic (H_2O_2 and •OH) and hydrophobic ($•NO_2$ and •NO) RONS through AQP1. The results revealed that these RONS could all penetrate across the AQP1. The permeation free energy barriers for •OH and •NO were lower than that of H_2O_2 and $•NO_2$, indicating that these radicals may have easier access to the pore interior and interact with the amino acid residues of AQP1. We also studied the effect of RONS-induced oxidation of both the phospholipids and AQP1 on the transport of the above-mentioned RONS across AQP1. Both lipid and protein oxidation slightly increased the free energy barrier for H_2O_2 and $•NO_2$ permeation, while for •OH and •NO, we did not observe a strong effect of oxidation (see Fig. 2). Hence, we suggest that the hydrophilic species enter the cell through AQPs, as they experience a clearly higher barrier through the lipid membrane, especially in the native lipid bilayer. On the other hand, the hydrophobic RONS most likely permeate through the membrane, where lipid oxidation can take place, as their barrier through AQP is higher (see [21] for more details).

Our next study consists of two parts. In a first part, we investigated the permeation of cystine across three model systems of xCT, the light subunit of the xC^- antiporter responsible for the cystine and glutamate translocation [22]. We considered the outward facing, occluded and inward facing configurations of xCT, applying a combination of targeted MD [24], steered MD [25] and classical MD as well as umbrella sampling [26] simulation techniques. We found the key role of amino acid residues in the outward facing, occluded and inward facing configurations responsible for the uptake, transport and release of cystine. The calculated free energy profiles of cystine across the outward facing, occluded and inward facing structures showed the energy minimum of cystine to be higher in the inward facing conformation, indicating that a lower energy is required for the release of cystine from the antiporter [22].

The second part was dedicated to the effect of plasma oxidation on the extracellular cystine uptake by the xCT [23], which is important for cancer therapy. We considered the outward facing configuration of xCT, and to study the effect of oxidation, we oxidized the Cys_{327} residue, located in the vicinity of the extracellular milieu. Our simulation results showed that oxidation of Cys_{327} results in a free energy barrier for cystine translocation, thereby blocking the access of cystine to the substrate binding site of the outward facing system. The obtained results indicated that the oxidation of Cys_{327}, by e.g. cold atmospheric plasma, is more effective in inhibiting the xC^- antiporter than the mutation of this amino acid to Ala [23].

IV. Conclusions

In this paper, we provided an overview of our latest simulation results that may help to gain more insight in the underlying mechanisms of the noticeable rise of cold atmospheric plasma-induced RONS in cancer cells, thereby improving our understanding on the role of integral membrane proteins in the selective anticancer activity of cold atmospheric plasma.

In particular, the simulation results on the RONS permeation across AQP revealed that oxidation of both the phospholipids and AQP1 protein does not strongly affect the permeation of •OH and •NO radicals across AQP1, whereas it slightly increases the free energy barrier for H_2O_2 and $•NO_2$, which we attributed to the sizes of these species [20, 21]. Both lipid and protein oxidation influences the shape of the free energy profiles of all RONS, as well as the barrier heights for H_2O_2 and $•NO_2$. Thus, these out comes provide molecular level insight into the role of AQP, which is often more expressed in cancer cells.

Our simulation results on the cystine uptake by the xCT protein revealed that oxidation of xCT protein leads to a free energy barrier for cystine translocation, thereby blocking the access of cystine to the substrate binding site of the outward facing system [22,23]. Hence, the inhibition of the xCT subunit of the xC^- antiporter may lead to Cys starvation in some cancer cells, eventually resulting in cancer cell death.

Acknowledgements. The computational work was carried out using the Turing HPC infrastructure of the CalcUA core facility of the Universiteit Antwerpen, a division of the Flemish Supercomputer Centre VSC, funded by the Hercules Foundation, the Flemish Government (department EWI) and the University of Antwerp.

References

[1] M. Keidar, R. Walk, A. Shashurin, P. Srinivasan, A. Sandler, S. Dasgupta, R. Ravi, R. Guerrero-Preston, B. Trink. Br. J. Cancer **105**, 1295 (2011).

[2] M. Wang, B. Holmes, X. Cheng, W. Zhu, M. Keidar, L.G. Zhang. PloS One **8**, e73741 (2013).

[3] F. Utsumi, H. Kajiyama, K. Nakamura, H. Tanaka, M. Hori, F. Kikkawa. Springer Plus **3**, 398 (2014).

©Academy of Sciences of Republic of Uzbekistan, 2022

[4] S.J. Kim, T. Chung. Sci. Rep. **6**, 20332 (2016).
[5] H.-R. Metelmann, C. Seebauer, V. Miller, A. Fridman, G. Bauer, D.B. Graves, J.-M. Pouvesle, R. Rutkowski, M. Schuster, S. Bekeschus, K. Wende, K. Masur, S. Hasse, T. Gerling, M. Hori, H. Tanaka, E.H. Choi, K.-D. Weltmann, P.H. Metelmann, D.D. von Hoff, T. von Woedtke. Clin. Plasma Med. **9**, 6 (2018).
[6] E.A. Ratovitski, X. Cheng, D. Yan, J.H. Sherman, J. Canady, B. Trink, M. Keidar. Plasma Process. Polym. **11**, 1128 (2014).
[7] J.Y. Kim, J. Ballato, P. Foy, T. Hawkins, Y. Wei, J. Li, S.-O. Kim. Biosens. Bioelectron. **28**, 333 (2011).
[8] X. Lu, M. Keidar, M. Laroussi, E. Choi, E.J. Szili, K. Ostrikov. Mater. Sci. Eng.: R **138**, 36 (2019).
[9] Y. Gorbanev, A. Privat-Maldonado, A. Bogaerts. Anal. Chem. **90**, 13151 (2018).
[10] U. Förstermann, W. C. Sessa. Eur. Heart J. **33**, 829 (2011).
[11] D. Tousoulis, A.-M. Kampoli, C. Tentolouris, Nikolaos Papageorgiou, C. Stefanadis. Curr. Vasc. Pharmacol. **10**, 4 (2012).
[12] M. Ishaq, M.D.M. Evans, K. Ostrikov. BBA-Mol. Cell Res. **1843**, 2827 (2014).
[13] M. Ishaq, S. Kumar, H. Varinli, Z.J. Han, A.E. Rider, M.D.M. Evans, A.B. Murphy, K. Ostrikov. Mol. Biol. Cell **25**, 1523 (2014).
[14] S.J. Kim, H.M. Joh T.H. Chung. Appl. Phys. Lett. **103**, 153705 (2013).
[15] D.B. Graves. Plasma Process. Polym. **11**, 1120 (2014).
[16] G. Bauer. Redox Biol. **6**, 353 (2015).
[17] D. Yan, A. Talbot, N. Nourmohammadi, J.H. Sherman, X. Cheng, M. Keidar. Biointerphases **10**, 040801 (2015).
[18] E.J. Szili, S.-H. Hong, R.D. Short. Biointerphases **10**, 029511 (2015).
[19] J. Van der Paal, E.C. Neyts, C.C.W. Verlackt, A. Bogaerts. Chem. Sci. **7**, 489 (2016).
[20] M. Yusupov, D. Yan, R.M. Cordeiro, A. Bogaerts. J. Phys. D: Appl. Phys. **51**, 125401 (2018).
[21] M. Yusupov, J. Razzokov, R.M. Cordeiro, A. Bogaerts. Oxid. Med. Cell Longev. **2019**, 1 (2019).
[22] M. Ghasemitarei, M. Yusupov, J. Razzokov, B. Shokri, A. Bogaerts. Arch. Biochem. Biophys. **664**, 117 (2019).
[23] M. Ghasemitarei, M. Yusupov, J. Razzokov, B. Shokri, A. Bogaerts. Arch. Biochem. Biophys. **674**, 108114 (2019).
[24] P. Ferrara, J. Apostolakis, A. Caflisch. J. Phys. Chem. B **104** (18), 4511 (2000).
[25] J.S. Patel, A. Berteotti, S. Ronsisvalle, W. Rocchia, A. Cavalli. J. Chem. Inf. Model. **54** (2), 470 (2014).
[26] J. Kästner. Wiley Interdiscip. Rev.: Comput. Mol. Sci. **1** (6), 932 (2011).

Плазма орқали оксидлашнинг оқсилларга таъсири: Молекуляр даражадаги моделлаштириш

М. Юсупов[1,2,3], М. Ғасемитарий[4], С. Мирзаев[2], У. Халилов[1,2,3]

[1] ТИҚХММИ Миллий тадқиқот университети, 100000, Тошкент, Ўзбекистон
[2] У.А. Арифов номидаги Ион-Плазма ва Лазер Технологиялари Институти, ЎзРФА, 100125, Тошкент, Ўзбекистон
[3] Антверпен университети, 2610, Антверпен, Бельгия
[4] Шариф Технология Университети, Техрон 14588-89694, Эрон

Сўнгги йилларда саратонни даволашда совуқ атмосферик плазмадан фойдаланиш плазма амалиётининг энг қизиқарли йўналишларидан бирига айланди. Кўп сонли тадқиқотларнинг истиқболли натижаларига қарамасдан, плазманинг саратон ҳужайраларига таъсирини тушунтирувчи асосий механизмларнинг ҳали ҳам ноаниқлиги чуқурроқ фундаментал тадқиқотлар ўтказилишини тақозо қилади.

Ушбу ишда совуқ атмосферик плазма муҳитида ҳосил бўлган реактив кислород ва азот зарраларининг саратон ҳужайрасидаги махсус оқсиллар билан ўзаро таъсир механизмларини ўрганиш учун олиб борилган сўнгги моделлаштириш тадқиқотларимиз ҳақида қисқача шарҳ берилган. Хусусан, ушбу шарҳда асосий эътибор реактив плазма зарраларининг интеграл мембрана оқсиллари учун модел тизимлар сифатида қўлланилган аквапорин ва цистин/глутамат ташувчиси хСТнинг ўтказувчанлигига таъсирини тушунишга қаратилган. Моделлаштириш натижалари реактив плазма зарраларининг нормал ҳужайраларга нисбатан саратон ҳужайраларида сезиларли даражада кўпайишининг асосий механизмларини яхшироқ тушунишга ёрдам беради ва натижада совуқ атмосферик плазманинг саратонга танлаб таъсир ўтказишида мембрана оқсилларининг роли тўғрисидаги умумий тушунчаларни яхшилайди.

Калит сўзлар: совуқ атмосферик плазма, саратонни даволаш, реактив кислород ва азот, молекуляр динамика.

©Academy of Sciences of Republic of Uzbekistan, 2022