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Short communication

Identification of oligopeptides from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) non structural protein 8 (NSP8) and their similarities with type 1 angiotensin II receptor key sites

Ammar Achour [1]

I VS 14 Bis Rue de Mayenne, 94000 Creteil, France

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ABSTRACT

Coronavirus disease 2019 is associated with clinical symptoms including severe inflammatory syndrome and a higher expression of angiotensin II. As a pro-inflammatory mediator, the physiologic effects of angiotensin II are mediated by a G-protein coupled receptor, termed AT_1R. Following binding, AT_1R initiates the process of signal desensitization necessary to maintain cellular homeostasis. At the cellular level, this function occurs via the G protein-dependent signaling and the phosphorylation. We describe amino acids similarities between SARS COV-2 nonstructural protein (NSP8) which is associated with intracellular membranes and AT_1R key sites. Since abnormal activation of AT_1R receptor leads to a number of physiological disorders, we hypothesize that SARS COV-2 might further interfere with the angiotensin II receptor functions.

1. Introduction

Following the isolation of SARS-COV-2 virus and its identification of the causative of severe acute respiratory syndrome [1], many studies have been reported to explain the characteristics of the disease [2,3]. SARS-COV-2 infects epithelial and endothelial cells, neurons, microglia, and lung macrophages expressing angiotensin-converting enzyme 2 (ACE-2). Moreover, the virus infects also monocytes, macrophages, dendritic cells, and lymphocytes, leading to the development of cytokine storm [4]. The binding of the virus on ACE2 leads to a downregulation of ACE2 and a higher expression of angiotensin II (Ang II). The physiologic actions of Ang II is mediated by a G-protein coupled angiotensin II type 1 receptor (AT_1R). Upon agonist stimulation a rapid internalization and desensitization of AT_1R is observed. Ang II has been shown to activate nuclear factor kappa-B (NF-κB) through binding to AT_1 R [5]. This binding results in the over-expression of inflammation cytokines leading to lymphopenia [5,6]. SARS-CoV-2 is characterized by a positive single strand RNA genome [7]. The virus contains several structural proteins like spike, membrane, envelope, nucleoprotein and non-structural proteins. The open reading frame 1ab (ORF1ab) represents approximately 67% of the genome length [8]. It encodes a polyprotein which is processed into 16 nonstructural proteins (Nsp1–16) which are involved in virus processing and replication. As a non structural viral protein, NSP8 is produced in the first stage of the virus cycle prior genome replication. Therefore, it is characterized as a peptide cofactor of RNA polymerase complex [9]. It has been reported that SARS-CoV-2 non structural proteins match to human proteins [10]. NSP8 have previously been shown to affect immune response signaling in the SARS-CoV experimental model [11].

2. Materials and methods

We considered studying the similarity between the NSP8 and type-1 angiotensin II receptor (AT_1R). In this line of research, we searched for similarities between NSP8 (NCBI Reference Sequence: YP_009742615.1) and type-1 angiotensin II receptor (NCBI Reference Sequence: NP_000676). Using Blast program (NCBI Blast-Protein Sequence), we studied sequences similarities containing at least 9 amino-acid. We were surprised to find the following similarities in a region of NSP8 region (residues 36–96). In this study, we used Needleman–Wunsch algorithm (NW) where double dots indicate identical residues and single dots indicate similarities. The three nsp8 sequences were located in a region (36−181).

Abbreviations: a.a, amino acids; ACE-2, angiotensin-converting enzyme 2; Ang II, angiotensin II; AT_1R, angiotensin II type 1 receptor; Cpp, cell penetrating peptide; Covid 19, Coronavirus disease 2019; LPS, lipopolysaccharides; NSP8, non structural protein 8; ORF1ab, open reading frame 1ab; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

E-mail address: ammar.achour@wanadoo.fr.
[1] ORCID: 0000-0002-2862-2149

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3. Results

3.1. Similarity between NSP8 (36–46) and AT_1R (137–146) peptides

The nsp8 KLKKSLNVAK (36-46) peptide shares 90% similarity (NW score: 23, Identities: 40%, Positives: 90%, Gaps: 0%) with type 1 AT_1 receptor peptide (137–145) RLRRTMLVAK which is located in the N-terminal part of the second intracellular loop (ICL) (Fig. 1).

R L R R T M L V A K (residues 137-146) / type-1 angiotensin II receptor.

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K L K K S L N V A K(residues 36-46) / Non-structural protein 8.

This receptor peptide consists in 2 parts. The terminal LVAK sequence (143–146) is integrated in the cell membrane whereas the (137–142) sequence RLRRTM is located in a transmembrane region corresponding to a second intracellular loop. It is characterized by the presence of several basic residues, such as **R**^{137}, **R**^{139}, and **R**^{140} in the C-terminal region of the second ICL. These peptides are localized in a LPS-binding peptide site (135–140 a.a) which is characterized by chemical studies [12]. These amino acids are required for G protein activation as previously reported [13]. Consequently, this binding might prevent the coupling to G-alpha-protein of the AT_1R. These 3 amino acids share similarities with **K**^{36}, **K**^{38} and **K**^{39} of the NSP8 peptide. It is noteworthy to indicate that a part of the peptide (KLKKSLNV) was described as a potential target of autoimmunity [10]. In this report, the nsp8 peptide (AVANGDSEVVLKKLKKSLNV) was suggested to be an autoimmunity component caused by SARS-CoV-2 by sharing homology with a sequence derived from a ubiquitous self antigen [10].

3.2. Similarity between NSP8 (89-97) and AT_1R (216-224) peptides

The nsp8 TMLFTMLRK sequence (89-97) is located in a helix type domain as characterized by secondary structure study. It shares 77% similarity (NW score: 19, Identities: 33%, Positives: 77%, Gaps: 0%) with type 1 Ang II receptor TLIWKALKK peptide (216–224) which is located within a helical structure as an intracellular loop 3 in the five transmembrane region (Fig. 1).

T L I W K A L K K (residues 216-224) / type-1 angiotensin II receptor.

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T M L F T M L R K (residues 89-97) / Non-structural protein 8.

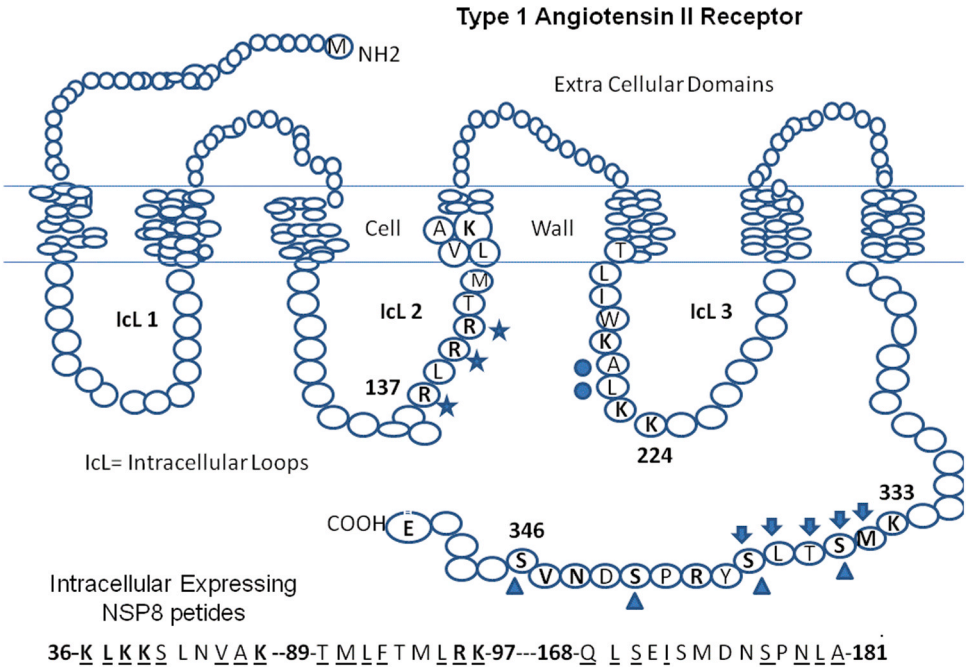

Fig. 1. Schematic representation of AT1 receptor key sites and their similarities with oligopeptides from (SARS-CoV-2) NSP8 protein. Bold character (**K,R**): indicates basic residues, ★: amino acids within LPS-binding site required for G protein activation, ●: amino acids required for Gq-mediated signaling, receptor internalization, and ERK activation, ⬇: amino acids involved in G protein-dependent and G protein-independent signaling, ▲: cytoplasmic tail amino acids involved in the agonist-induced desensitization, (a.a) underlined NSP8 a.a which share similarity with AT_1R.

It was reported that the substitutions (**L**^{222}/V) in the N-terminal and C-terminal portions of the third intracellular loop could modulate coupling to G proteins. Among specific amino acids in the amino-terminal region of ICL 3, mutation of **L** ^{222} markedly decreased the affinity for Ang II. Deletion of **A**^{221} and **L**^{222} interfered with Gq-mediated signaling, receptor internalization, and ERK activation [14]. These are key amino acid residues in AT_1 receptor for Ang II stimulation of p42/44 MAPK activation and are known to interact with any GPCR [15]. **L**^{222} is identical to **L**^{95} NSP8 amino acid. More interestingly, the NSP8 TMLFTMLRK peptide was classified as a cell penetrating peptide (cpp) since it contains (M^{90} L^{91} **FTM** ^{94}**LRK**) amino acids [11]. Amino acids (**I**^{218} **W**^{219} **LKK** ^{222}**)** similarities were observed in AT_1 R peptide which is located in a helical structure. Interactions between NSP7 and NSP8 reveal that the binding region for NSP7-NSP8 heterodimer is conserved. It was reported that residues of SARS-CoV-2 NSP8 (**M**^{87}, **M**^{90}, **L**^{91}, **M**^{94} and NSP7 (V^{6}, C^{8}, V^{12}, and V^{16}) can form a hydrophobic core as observed in SARS-CoV-1 NSP8 model leading optimal replication and transcription [11].

3.3. Similarity between NSP8 (168-181) and AT_1R (333-346) peptides

The nsp8 sequence QLSEISMDNSPNLA (residues 168–181 share 64% similarity with type 1 AC2 receptor peptide KMSTLSYRPSDNVS which is located in a carboxyl-terminal cytoplasmic domain (residues 333–346) (NW score: 18, Identities: 29%, Positives: 64%, Gaps: 0%) (Fig. 1).

K M S T L S Y R P S D N V S (residues 333–346) / type-1 angiotensin II receptor.

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Q L S E I S M D N S P N L A (residues 168–181) / Non-structural protein 8.

Endocytosis of the AT_1R receptor requires phosphorylation within the serine/threonine-rich segment of the carboxyl terminus. It was reported that substitutions within the carboxyl-terminal region contribute strongly to receptor internalization [15]. Studies using amino acid mutations or deletions reported that **M**^{334}, **S**^{335}, **L**^{337} and **S**^{338} are involved in G protein-dependent and G protein-independent signaling. Similar **L**^{169}, **S**^{170}, **I**^{172} and **S**^{173} amino acids are also observed in NSP8. Studies suggest that the carboxy-terminal tail is important for interaction with β-arrestins, and that it plays a minor role in G protein-dependent signaling. Moreover, it was indicated that Ser/Thr phosphorylation sites located between **S**^{328} and **S**^{347} in the cytoplasmic tail are important for the agonist-induced desensitization [15]. By comparison, **S**^{170}, **S**^{173} and **S**^{177} amino acids were also localized in NSP8.

4. Discussion

Nsp8 is thought to be part of the viral replication complex, which is associated with intracellular membranes. It is located in cytoplasmic foci, largely perinuclear. Late in infection, it merges into confluent complexes including nsp7, nsp9 and nsp10**.** The non structural viral proteins are produced prior to generation of double strand RNA products during virus genome replication. Moreover, it was recently reported that NSP8 is sufficient to suppress protein integration into the cell membrane. By interfering with protein expression in the cell membrane NSP8 can suppress the interferon response [16].

Following binding to angiotensin II, AT_1 R is submitted to a rapid internalization and desensitization to maintain cellular homeostasis [17]. The binding results in the over-expression of inflammation cytokines called “cytokines storm” observed in covid-19 disease [5,6]. The G-protein coupled receptor (AT_1R) is located in the extracellular, transmembrane and intracellular regions. The interaction of the receptor and ligand results in the activation of phospholipase C (PLC) via the coupling of the receptor with G proteins leading to expression of proinflammatory genes [5]. In this study, we show similarities between NSP8 and i) the N-terminal part of the second intracellular loop (ICL), ii) the C-terminal segment of the third intracellular loop and iii) in the carboxyl-terminal cytoplasmic domain. The C-terminal region of the second ICL contains basic residues localized in a LPS-binding peptide. These amino acids are required for G protein activation [13]. The third cytoplasmic loop of AT_1R receptor is implicated in the specificity of receptor coupling to G proteins including Gq-mediated signaling, receptor internalization, and ERK activation [14,15]. The carboxyl-terminal cytoplasmic domain is involved in G protein-dependent and G protein-independent signaling [15]. Then, the observed similarities between NSP8 and AT_1R may lighten the function of this receptor in sars-cov 2 infection. By such similarities, we suggest that NSP8 might interfere with protein G modulation [17]. A major factor regulating angiotensin II receptor function is the rapid desensitization following agonist stimulation. Whether the process of signal desensitization by phosphorylation of G-protein-coupled receptor is impaired or activated by NSP8 remains to be determined. The identification of NSP8 domains sharing similarity with the AT_1 receptor could increase our knowledge of how the receptor is regulated in intracellular following infection. Future studies will investigate the physiological importance of the NSP8 motifs role in the sensitization of AT_1R.

In summary our study is the first to show similarities between sars cov- 2 NSP8 and AT_1R sites necessary to protein G signaling and receptor modulation. Although it remains unknown what, if any, role these similarities have in virus-infected cells, it suggests that they may provide a comprehension of the pathogenesis of COVID-19. Taken together, our results provide information to explore the physiological actions of NSP8 in covid-19 disease.

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Conflict of interest statement

The author has no conflict of interest to declare.

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