UDC 57.577 https://doi.org/10.15407/biotech16.02.032

STRUCTURAL PATTERNS OF IVERMECTIN ALLOSTERIC INTERACTION WITH GLUTAMATE-GATED CHLORIDE CHANNEL OF *Caenorhabditis elegans*

Y. O. KUSTOVSKIY[1,2], *A. I. YEMETS*[1,2]

[1]Institute of Food Biotechnology and Genomics
of the National Academy of Sciences of Ukraine, Kyiv
[2]National University of Kyiv Mohyla Academy, Ukraine

E-mail: ykustovskiy@gmail.com

Received 2023/03/30
Revised 2023/04/04
Accepted 2023/04/28

The drug repurposing, which is the search of new applications for known bioactive compounds, has apparent benefits (e.g., cost efficiency, development risks, and time reduction) as compared to the traditional *de novo* drug design making it common for different research fields including the biological drug discovery [1]. The nematocide and insecticide ivermectin (IVM) is considered a wide spectrum agent, which makes it a perspective candidade for repurposing [2]. Although the structure-activity relationships of IVM interactions with its traditional targets of Cys-loop receptors family are studied [3], their structural patterns have not been described yet. The knowledge of structural patterns, which are groups of residues related spatially and by physico-chemical properties, with high affinity for IVM functional groups can be used to find local similarities in active sites of IVM potential targets when the direct alignment of sites cannot be implemented due to the evolutionary distinction between known and potential targets [4].

Aim. To determine the structural patterns of IVM allosteric interaction with residues of its binding site located in the transmembrane domain of α-homopentameric glutamate-gated chloride channel (GluClα) of *Caenorhabditis elegans*.

Methods. To consider different conformational states of IVM binding site two complexes of IVM bound to *C. elegans* GluClα (each with five site conformations) with identifiers 3RHW (https://doi.org/10.2210/pdb3RHW/pdb) and 3RIF (https://doi.org/10.2210/pdb3RIF/pdb) were obtained from PDB [5]. The structures were examined in Analyzer Mode of SeeSAR v.12.1.0, in which contributions of IVM atoms into the complex affinity and their interactions with site structural patterns were determined for each site conformation using the HYDE scoring function [6]. The residues belonging to identified structural patterns were classified by their properties using the Taylor's classification of amino acids [7].

Results and Discussions. The binding site of IVM on cys-loop receptors including GluClα is located in the interface between transmembrane domains of (+) and (−) subunits and is formed by M2, M3, and M2-M3 of (+) subunit and M1 of (−) subunit (Fig. 1, *A*). As it is demonstrated on the Fig. 1, *B*, IVM is composed of the 16-membered heteromacrocyclic ring fused with the spiroketal and benzofuran groups, and linked with the disaccharide group.

According to the results, the benzofuran group is critical for IVM recognition and binding: it interacts with the T-A-S-N-D-I-L-Q-I-P pattern, which is formed by T257, A258, S260, and N264 of M2, D277 and I280 of M3 of (+) subunit and L218, Q219, I222, P223 of M1 of (−) subunit (Fig. 2, *A*). Due to the size and hydrophobicity of macrocycle, its different parts interact with residues of all site-forming structural elements mentioned above resulting in the V-I-G-A-M and I-V-D-L patterns demonstrated on the Fig. 2, *B, C*. While the V-I-G-A-M pattern is formed by the residues of (+) subunit (V278, I280, G281, A282, and M284 of M3), the I-V-D-L pattern contains residues of both subunits: I273 of M2-M3, D277 and V278 of M3 of (+) subunit and L218 of M1 of (−) subunit. Finally, the spiroketal group interacts with M-T-F-C-M-I of (+) subunit (M284, T285, and F288 of

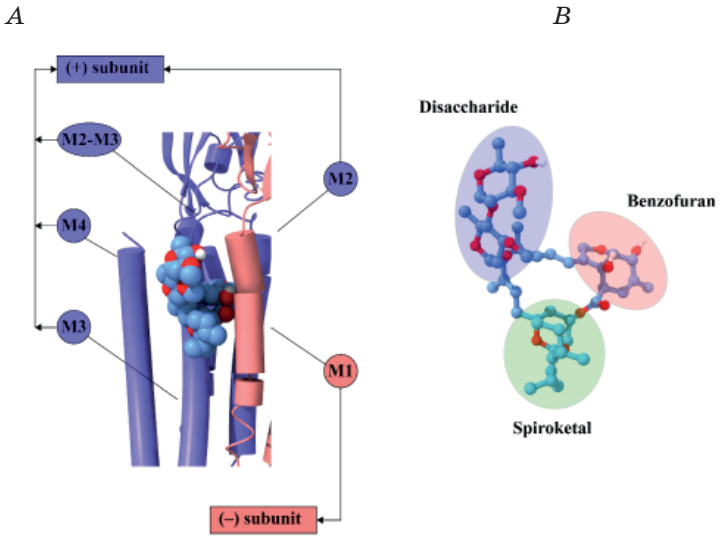

Fig. 1. **The binding site of IVM on GluClα (*A*) and the bioactive conformation of IVM (*B*) visualized using the UCSF ChimeraX v. 1.5 [8]**

M3) and (–) subunit (C225, M226, and I229 of M1) (Fig. 2, *C*). As opposed to other functional groups, the disaccharide is located outside of the binding site pocket. It interacts with I273 of M2-M3 of (+) subunit and L217, L218, and I222 of M1 of (–) subunit; however, considering that these residues are not united spatially, no pattern for the disaccharide can be determined based on the structural information which was analyzed. The determined structural patterns of IVM allosteric interaction with GluClα can be used in search of IVM binding site on its potential targets, in the development of hypotheses of IVM binding to identified sites, and to rationalize the drug design of new GluCl ligands.

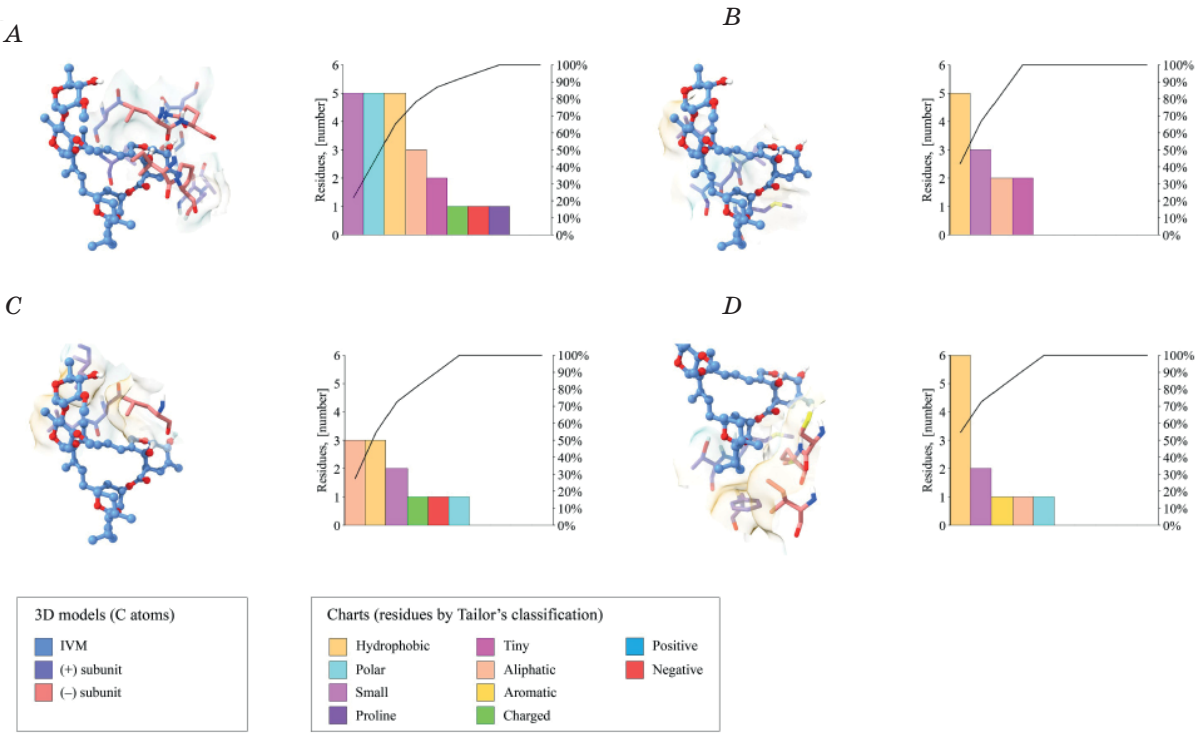

Fig. 2. **The identified structural patterns with high affinity for IVM functional groups:**
A — T-A-S-N-D-I-L-Q-I-P; *B* — V-I-G-A-M; *C* — I-V-D-L; *D* — M-T-F-C-M-I:
the physico-chemical properties of their residues, which were determined based on the Taylor's classification of amino acids, are represented on the respective graphs

Conclusions. The structural patterns with high affinity for IVM functional groups have been determined based on the results of HYDE assessment and visual analysis of IVM-GluClα complexes and the possible implementations of patterns knowledge have been described. The identified patterns can be further corrected and extended using the structural information of other IVM targets deposited in PDB.

Key words: ivermectin; drug repurposing; *Caenorhabditis elegans*; glutamate-gated chloride channels; *in silico* molecular modeling.

Authors' contribution. The YOK performed analysis of structural information and wrote the first draft. AIY formulated the concept of research and supervised the project. Both authors contributed equally to final writing.

Funding source. The work was funded by the National Academy of Sciences of Ukraine (КПКВК 6541230).

REFERENCES

1. *Mittal N., Mittal R.* Repurposing old molecules for new indications: defining pillars of success from lessons in the past. *Eur. J. Pharmacol*. 2021, 912 (4), 1–12. https://doi.org/10.1016/j.ejphar.2021.174569
2. *Martin R. J., Robertson A. P., Choudhary S*. Ivermectin: an anthelmintic, an insecticide, and much more. *Trends Parasitol*. 2021, 37 (1), 48–64. https://doi.org/10.1016/j.pt.2020.10.005
3. *Chen I. S., Kubo Y*. Ivermectin and its target molecules: shared and unique modulation mechanisms of ion channels and receptors by ivermectin. *J. Physiol*. 2018, 596 (10), 1833–1845. https://doi.org/10.1113/jp275236
4. *Salentin S., Haupt V. J., Daminelli S., Schroeder M*. Polypharmacology rescored: protein–ligand interaction profiles for remote binding site similarity assessment. *Prog. Biophys. Mol. Biol*. 2014, 116 (2–3), 174–186. https://doi.org/10.1016/j.pbiomolbio.2014.05.006
5. *Hibbs R. E., Gouaux E*. Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature*. 2011, 474 (7349), 54–60. https://doi.org/10.1038/nature10139
6. *Schneider N., Lange G., Hindle S., Klein R., Rarey M*. A consistent description of HYdrogen bond and DEhydration energies in protein–ligand complexes: methods behind the HYDE scoring function. *J. Comput. Aided Mol. Des*. 2013, 27 (1), 15–29. https://doi.org/10.1007/s10822-012-9626-2
7. *Valdar W. S. J*. Scoring residue conservation. *Proteins*. 2001, 48 (2), 227–241. https://doi.org/10.1002/prot.10146
8. *Pettersen E. F., Goddard T. D., Huang C. C., Meng E. C., Couch G. S., Croll T. I., Morris J. H., Ferrin T. E*. UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci*. 2021, 30 (1), 70–82. https://doi.org/10.1002/pro.3943