Human Vitamin K Epoxide Reductase as a Target of its Redox Protein

Julie Ledoux, Maxim Stolyarchuck, Luba Tchertanov

Human Vitamin K Epoxide Reductase as a Target of its Redox Protein

Julie Ledoux[1] and Maxim Stolyarchuck[1] Luba Tchertanov[1]

Université Paris-Saclay, ENS Paris-Saclay, Centre Borelli, 4 avenue des Sciences, 91190 Gif-sur-Yvette, France
julie.ledoux@ens-paris-saclay.fr

Abstract. Human Vitamin K epoxide reductase (hVKORC1) is a key enzyme to reduce vitamin K. Such function requires activation of the enzyme by a redox partner delivering reducing equivalents through thiol-disulphide exchange reactions. The activation process represents a first and less studied step in hVKORC1 vital cycle, involving the oxidised luminal loop (L-loop) and a reduced thioredoxin protein (Trx), which is yet undefined for hVKORC1. A careful *in silico* study, based on molecular dynamic (MD) simulations of hVKORC1 in oxidised state, and a comparative analysis of four Trx proteins - protein disulphide isomerase (PDI), endoplasmic reticulum oxidoreductase (ERp18), thioredoxin-related transmembrane protein 1 (Tmx1) and thioredoxin-related transmembrane protein 4 (Tmx4)), viewed as the most probable reducers of hVKORC1 – in their sequence, secondary and tertiary structure, dynamics, intra-protein interactions and composition of the surface exposed to the target – provided the identification of putative recognition/binding sites on each isolated protein. PDI was suggested as the most probable hVKORC1 partner. By probing the alternative orientation of PDI with respect to hVKORC1, two PDI-VKOR models were proposed and one of them considered as precursor for thiol-disulphide exchange reactions.

Keywords: hVKORC1; Trx-fold redox proteins · protein folding; intrinsic disorder · modular protein · molecular recognition · thiol–disulphide exchange · protein–protein interactions · PDI–hVKORC1 complex · 3D modelling · molecular dynamics

The human vitamin K epoxide reductase (hVKOR) hVKORC1 is an endoplasmic reticulum (ER)-resident transmembrane protein reducing vitamin K inside a membrane-embedded cysteine-containing redox centre [8]. Such activity requires the cooperation of VKOR with a redox partner delivering reducing equivalents through thiol-disulphide exchange reactions, involving a disulphide bridge from the extended luminal loop (L-loop) of VKOR [5]. The activation process represents a first and less studied step in VKOR vital cycle. The physiological redox partner of hVKORC1 remains uncertain. Four human redoxin proteins (Trx) protein disulphide isomerase (PDI), endoplasmic reticulum oxidoreductase (ERp18), thioredoxin-related transmembrane protein 1 (Tmx1) and thioredoxin-related transmembrane protein 4 (Tmx4) – were suggested as the most probable

H-donors of hVKORC1 [6]. In addition, the structure of hVKORC1 L-loop is not credibly characterised. Consequently, deciphering the molecular origins of hVKORC1 recognition by an unknown redox protein is not a trivial task.

We suggested that an accurate *in silico* study of Trxs and hVKORC1 as isolated proteins would provide useful information for the development of putative Trx-VKOR complexes. Quantitative metrics and qualitative estimations can shed new light on the target (hVKORC1) features and peculiarities of redox proteins (Trx). Such information may help in predicting (i) the protein fragments participating in VKORC1 recognition by a Trx and (ii) the most probable partner of VKORC1.

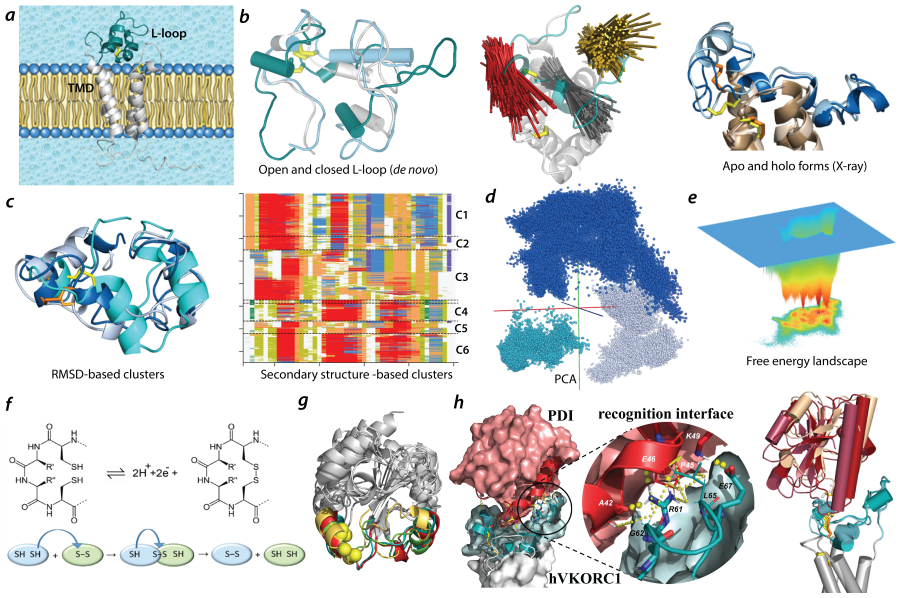

Fig. 1. 3D model of VKORC1 and its complex with redox protein (a) VKORC1 inserted into membrane and surrounded by water molecules. The helically folded L-loop shows structural and conformational disorder in *de novo* model (left and middle) and in X-ray structures. (b) Clustering of L-loop conformations using RMSD and secondary structures. (d) Projection of MD conformations on the three principal components determined by principal component analysis (PCA). (e) Free energy landscape of L-loop conformations defined on two primary reaction coordinates, RMSD and radius of gyration (Rg). (f) Mechanism of disulphide exchange between Trx and a target. (g) Superimposed 3D structures of Trx-fold proteins with cysteine residue as yellow balls. Regions that are potentially involved in target recognition and/or electron transfer reaction are differentiated by colour. (h) Modelling of human PDI–hVKORC1 complex (left) and reproduced results by protein-protein docking (HADDOCK) (right).

First, conformational features of hVKORC1 and L-loop, the principal platform of hVKORC1 for Trx recognition, scaffolding and intermolecular thiol-disulphide exchange reactions, were characterised by extended molecular dynamics simulations (MD) of a de novo model [1, 7] and crystallographic structures [3] of the enzyme in oxidized state. This study clearly showed that (i) L-loop is an intrinsically disordered region, and (ii) hVKORC1 is a modular protein composed of the structurally stable transmembrane domain (TMD) crowned by the disordered L-loop [2] (Figure 1 a-e). Indeed, the structurally well conserved TM helices, varied slightly only at their ends, show a cooperative drift typical for transmembrane domains rigidified by the stable non-covalent interactions. In contrast, L-loop exhibits an unstable helical fold represented by reversible transient - and 310-helices linked by flexible coils, offering L-loop a great conformational diversity, from compact 'globule-like' shape (closed form) to extended (open form) (Figure 1, b-c).

Such modular architecture of hVKORC1 provides (i) excellent conformational plasticity required for specific adaptation over recognition by redox protein, activation process and catalysis, and (ii) easy and exact reproducibly of hVKORC1 metastable intermediates during repeated enzymatic cycles. Those qualities are strictly required for hVKORC1 activities [4]. Structure and conformations of the disordered L-loop are better described in terms of free energy than conventional methods such as clustering.

Secondly, focusing on Trx-fold proteins, probable hVKORC1 redox partners, we found that, despite similar architecture, each redox partner has its own sequence-dependent dynamical features. Further analysis identified PDI as the most probable redox partner of hVKORC1 (Figure 1f-h) [7]. By probing PDI alternative orientations with respect to hVKORC1, two models of noncovalent complex were proposed. One of them was considered as functionally related model and postulated as the first precursor to probe thiol–disulphide exchange reaction. This predicted complex, formed by hVKORC1 and PDI, was further reproduced by docking trials (protein-protein docking with HADDOCK) [2].

Finally, results obtained for hVKORC1 simulated in different environment (water/membrane) and simulation methods (conventional and accelerated) indicated that, for **in silico** study of hVKORC1 and its complexes, the membrane is probably not necessary, and the cleaved L-loop, simulated as isolated polypeptide, reflects its properties when fused to the transmembrane domain. Therefore, it may be used to study hVKORC1 recognition by its redox protein. Extension of these conclusions for experimental studies of hVKORC1 requires their empirical validation.

References

1. Chatron, N., Chalmond, B., Trouvé, A., Benoît, E., Caruel, H., Lattard, V., Tchertanov, L.: Identification of the functional states of human vitamin k epoxide reductase from molecular dynamics simulations **7**(82), 52071–52090. https://doi.org/10.1039/C7RA07463H, http://xlink.rsc.org/?DOI=C7RA07463H

2. Ledoux, J., Stolyarchuk, M., Bachelier, E., Tchertanov, L.: Human vitamin k epoxide reductase as a target of its redox protein . https://doi.org/In preparation
3. Liu, S., Li, S., Shen, G., Sukumar, N., Krezel, A.M., Li, W.: Structural basis of antagonizing the vitamin k catalytic cycle for anticoagulation **371**(6524), eabc5667. https://doi.org/10.1126/science.abc5667
4. Ma, B., Kumar, S., Tsai, C.J., Hu, Z., Nussinov, R.: Transition-state ensemble in enzyme catalysis: Possibility, reality, or necessity? **203**(4), 383–397. https://doi.org/10.1006/jtbi.2000.1097, https://linkinghub.elsevier.com/retrieve/pii/S002251930091097X
5. Rishavy, M.A., Usubalieva, A., Hallgren, K.W., Berkner, K.L.: Novel insight into the mechanism of the vitamin k oxidoreductase (VKOR) **286**(9), 7267–7278. https://doi.org/10.1074/jbc.M110.172213, https://linkinghub.elsevier.com/retrieve/pii/S0021925820519212
6. Schulman, S., Wang, B., Li, W., Rapoport, T.A.: Vitamin k epoxide reductase prefers ER membrane-anchored thioredoxin-like redox partners **107**(34), 15027–15032. https://doi.org/10.1073/pnas.1009972107, http://www.pnas.org/cgi/doi/10.1073/pnas.1009972107
7. Stolyarchuk, M., Ledoux, J., Maignant, E., Trouvé, A., Tchertanov, L.: Identification of the primary factors determining the specificity of human VKORC1 recognition by thioredoxin-fold proteins **22**(2), 802. https://doi.org/10.3390/ijms22020802, https://www.mdpi.com/1422-0067/22/2/802
8. Tie, J.K., Stafford, D.W.: Structure and function of vitamin k epoxide reductase **78**, 103–130. https://doi.org/10.1016/S0083-6729(07)00006-4