Identification and characterization of the vitamin K binding pocket in human VKORC1

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Introduction and Objective

Vitamin K Oxidoreductase Complex subunit 1 (VKORC1) catalyzes the reduction of vitamin K to sustain γ-carboxylation of the vitamin K dependent (VKD) coagulation factors (e.g. FIX) essential for their physiological activity. Defects in vitamin K recycling results in coagulopathies due to under-carboxylated VKD coagulation factors. Therefore, vitamin K reduction is essential for a well-functioning haemostasis although the binding site of vitamin K on VKORC1 is unknown. Thus, the aim of our study was to identify and characterize the vitamin K binding pocket.

Materials and Methods

We performed docking of vitamin K and membrane-embedded simulation on a homology based model of human VKORC1 (hVKORC1) to determine putative residues contributing to vitamin K1 quinone (K1) and vitamin K1 2,3-epoxide (K1>O) binding. To experimentally verify the *in silico* analysis we introduced structurally and biochemically non-conservative substitutions for theses amino acids in a bicistronic vector carrying cDNAs of *VKORC1* and coagulation factor *F9*. Amino acids K30, F55, Q78, N80, L120, I123, L124, and L128 were mutated to glycine in order to investigate interactions with the vitamin K naphtoquinone head (group A residues, yellow box in Fig. 1b, 1c). F87, Y88, L90, V112, and S113 were mutated to glycine (group B residues, green box in Fig. 1b, 1c) and G84 and G116 to phenylalanine (group C residues, magenta box in Fig. 1b, 1c) in order to characterize the incorporation of the isoprenoid chain of vitamin K. These variants were expressed in CRISPR/Cas9 genome engineered *VKORC1+VKORC1L1* double knockout HEK293 cells that do not possess endogenous vitamin K 2,3-epoxid reductase (VKOR) activity. This is essential to assess specific activity of co-expressed VKORC1 variants. Measured FIX activity served as reporter for VKOR activity and was normalized to VKORC1 protein expression.

Results

Docking of K1 and K1>O on our VKORC1 model revealed high ranked docking poses. The vitamin K naphthoquinone head groups are stabilized in close proximity of a short helix within the cytoplasmic loop by a combination of hydrophobic interactions and an aromatic stacking interaction with the phenyl side chain of Phe55 (Fig 1a-c). The isoprenoid chains of K1 and K1>O are predicted to be embedded in the hydrophobic transmembrane cavity within contact distances with aromatic as well as non-aromatic hydrophobic residues (Fig 1a-c). Introducing bulky side chains in close proximity to the vitamin K side chain binding region influenced proper insertion of its isoprenoid chain thereby affecting the activity of these variants (group C residues, Fig 1d). Substitutions with structurally small glycine in other regions also influenced activity of the specific variants most likely by diminishing the hydrophobic interactions (group A and B residues, Fig 1d). Furthermore, some mutated residues of group A showed low VKR activity but high VKOR activity indicating that while K1 and K1>O occupy similar hydrophobic pockets, subtle differences in their naphthoquinone head binding exist.

Conclusion

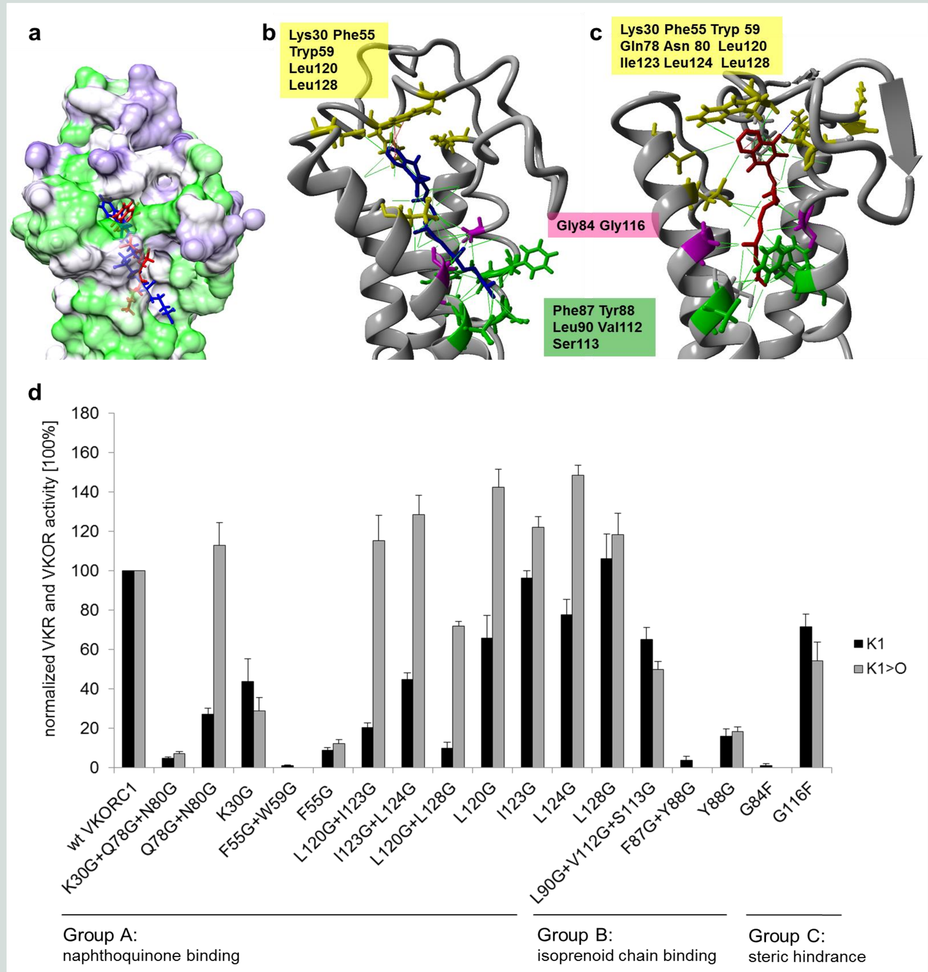
Using a combination of *in silico* and *in vitro* analysis we have been able to determine and characterize the vitamin K binding pocket on human VKORC1.

Fig.1: Mutation based characterization of *in silico* predicted K1 and K1>O binding residues of hVKORC1

a) Molecular surface description of the putative K1 and K1>O binding sites on hVKORC1 model. The molecular surface is colored based on surface hydrophobicity. Green color depicts maximum hydrophobic nature while purple represents the least hydrophobic areas. K1 and K1>O docked within hVKORC1 are depicted in red and blue colored stick forms.

b) A 100ns long simulation averaged structure of a selected docking pose of K1>O (blue) on hVKORC1 model (gray). The binding site residues participating in interactions with K1>O have been depicted in stick format. Group A: yellow, Group B: green and Group C: magenta (for group definitions refer to methods). The multiple colored lines depict different forms of interactions with hVKORC1 binding site residues i.e. Green: hydrophobic interactions, Red: π-π, Blue: Cation-π.

c) A 150ns long simulation averaged structure of a selected docking pose of K1 (red) bound to hVKORC1 model (gray). The binding site residues participating in interactions with K1 are depicted in colored stick forms as in panel (b). The multiple colored lines depict different forms of interactions with the hVKORC1 binding site residues as in (b).



d) Vitamin K quinone reductase (black bars) and vitamin K 2,3-epoxid reductase (grey bars) activities of hVKORC1 variants of group A, B, or C potentially involved in K1 or K1>O binding. Error bars, SD; n=4 replicates.

References

1. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hörtnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG, Müller CR, Strom TM, Oldenburg J. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature. 2004;427:537-41
2. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. Nature. 2004;427:541-4.
3. Li W, Schulman S, Dutton RJ, Boyd D, Beckwith J, Rapoport TA. Structure of a bacterial homologue of vitamin K epoxide reductase. Nature. 2010;463:507-12.

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