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Reversal of the Antithrombotic Properties of Quercetin-3-Rutinoside with the b' Domain of PDI in a Mouse Thrombosis Model

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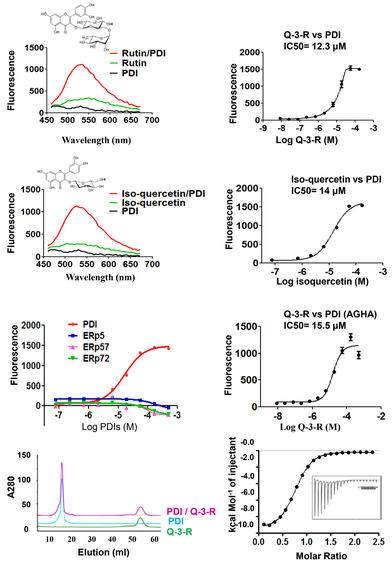
Introduction: We previously demonstrated that the inhibition of protein disulfide isomerase (PDI) activity blocks both platelet accumulation and fibrin generation. We identified quercetin-3-rutinoside (Q-3-R) as inhibitor of PDI and found it inhibits both platelet thrombus formation and fibrin generation in a mouse thrombosis model. However, the binding site of quercetin-3-rutinoside on PDI is unknown and strategies for reversing the effect of quercetin-3-rutinoside following potential bleeding complications during antithrombotic therapy are unexplored.

Method: The direct interaction of quercetin-3-rutinoside with PDI are established by fluorescence enhancement of quercetin-3-rutinoside with PDI, and the isothermal calorimetry measurement. Small angle X-ray scattering (SAXS) data were collected on the SIBYLS (Structurally Integrated Biology for Life Sciences) X-ray beam line. In vivo thrombus formation was induced by laser-induced injury to the cremaster arterioles and monitored by intravital microscopy.

Results:

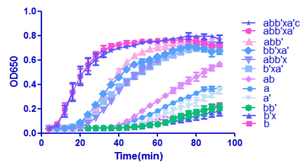
1. Q-3-R directly binds to PDI with a IC50 of 12.3 μM and 1:1 stoichiometry.



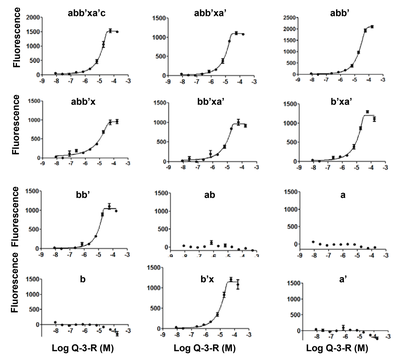
2. Generation of recombinant PDI fragments

Fragment	residue	MW
a	1-117	12959.5
b	118-218	11027.2
b'	219-331	13121.0
b'x	219-351	15479.7
a'	352-462	12442.0
ab	1-218	24026.8
bb'	118-331	24033.1
b'xa'	219-462	27907.8
abb'	1-331	37129.9
abb'x	1-351	39488.6
bb'xa	118-462	38917.0
abb'xa'	1-462	51916.6
abb'xa'c	1-491	55294.0

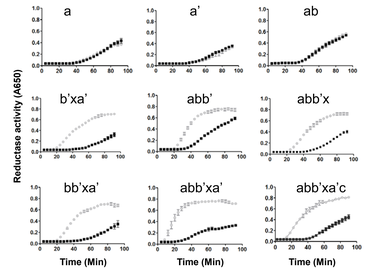
3. PDI b' domain enhances the activity of catalytic domains



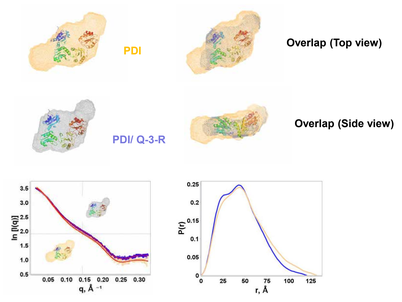
4. Q-3-R binds to the b' domain of PDI



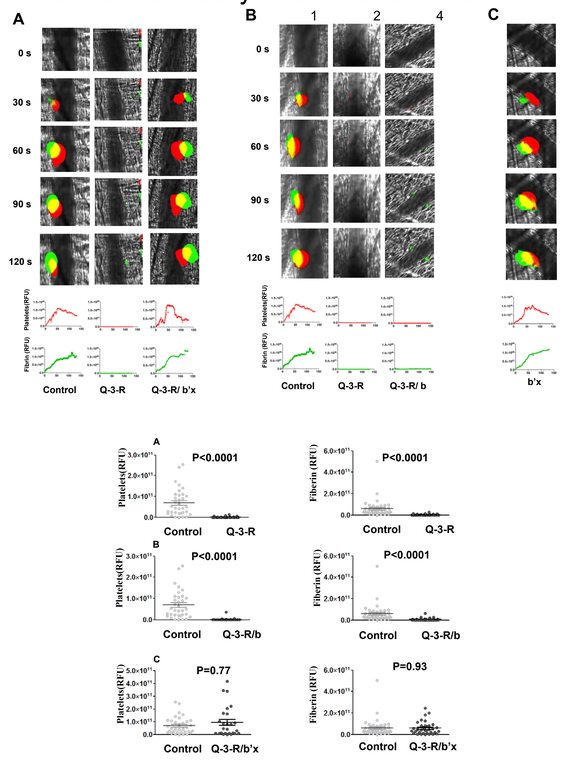
5. The reductase inhibition of Q-3-R on PDI is through binding of the b' domain.



6. SAXS measurement shows that PDI adapts a more compact conformation upon binding of Q-3-R.



7. The PDI b'x fragment rescues the inhibition of thrombus formation mediated by Q-3-R, but does not itself alone modify thrombus formation.



Conclusions: Quercetin-3-rutinoside binds directly to the b' domain of PDI. Such binding induce a conformational change in PDI to a more compact form and restricts the flexibility of the protein which is required for its function. The b' domain of PDI has the potential for use as an antidote to reverse the antithrombotic effect of quercetin-3-rutinoside in the event of bleeding complications.