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Exploring the structure-function relationship of heme binding to blood coagulation factor VIII based on peptide models

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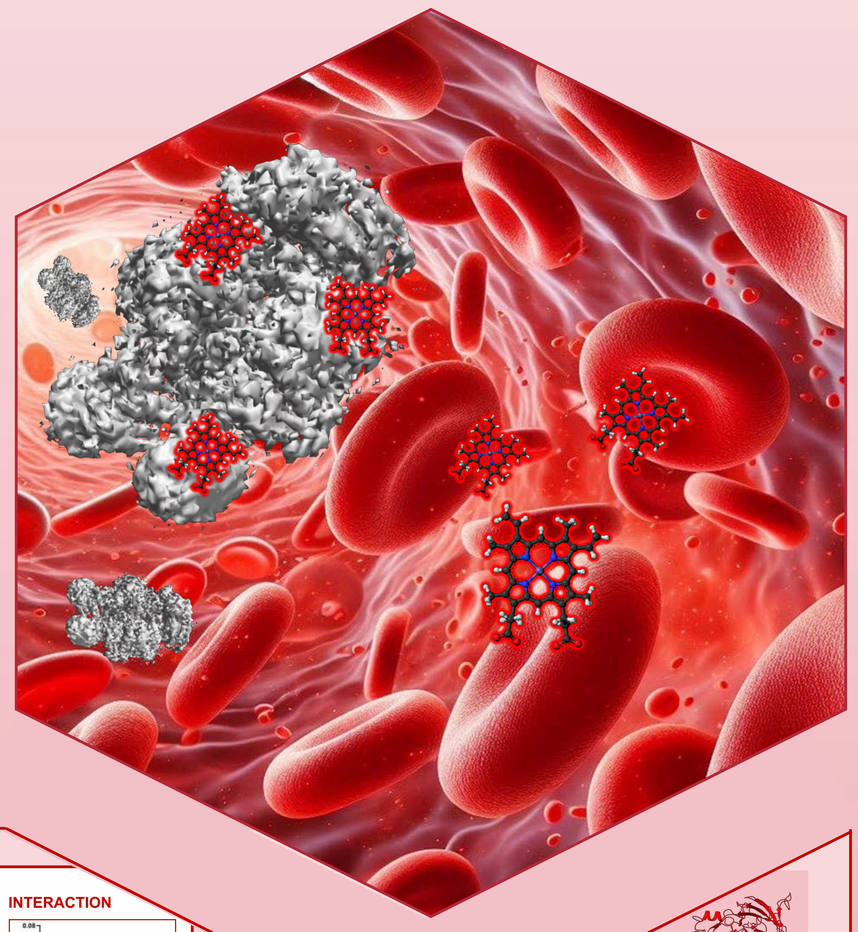

Background

Patients with hemophilia A often suffer from hemorrhage leading to massive accumulation of heme in, for example, the bleeding joint. To target the underlying deficiency of coagulation factor VIII (FVIII) and the associated complications, the protein is usually substituted. In this regard, it has been reported that direct FVIII injection into the bleeding site, meaning into the heme-rich environment, shows significantly fewer side reactions.[1] However, basic information about an interaction between FVIII and heme as well as the functional consequences thereof are still missing.[2,3] In the past, peptides arose as suitable models for the prediction of heme-binding proteins, mimicking potential surface-exposed Cys-, His- and/or Tyr-based heme-binding stretches, so-called "heme-binding motifs" (HBMs).[4] Spectroscopic characterization of the heme-binding capacity of protein-derived peptides allowed for the transfer onto protein level, as demonstrated by several proteins.[4,5] These results led to the establishment of a webserver, called "HeMoQuest", for the sequence-based prediction of HBMs in proteins.[4,6] Herein, we thus transferred the usage of protein-derived peptides as HBM models for the first time onto a procoagulant blood coagulation factor, namely FVIII, which is highly relevant in the therapy of hemophilia A patients.[7]

Results

Identification of heme-binding sites in FVIII by peptide models

The sequence of FVIII was screened for potential heme-binding motifs (HBMs) by using HeMoQuest (*http://131.220.139.55/SeqDHBM/*).[6]

Manual refinement (= exclusion of non-exposed motifs and combination of overlapping motifs) led to a selection of 17 potential HBMs in FVIII.[7] These motifs were synthesized as peptides following standard Fmoc/*t*Bu solid phase peptide synthesis. Heme binding to these FVIII-derived peptides (5-10 µM) was investigated by UV/Vis spectroscopy upon incubation with heme (increasing concentrations from 0.4 to 40 µM), which revealed four high affinity HBMs (**9**, **15**, **13**, and **17**) and one HBM (**16**) with moderate heme-binding affinity in the FVIII light chain as well as four HBMs (**1**, **3**, **4**, and **8**) with moderate affinity in the heavy chain. The heme-binding mode of select peptides was characterized by rRaman spectroscopy showing e.g., the presence of penta-coordinated complexes in case of peptides **9**, **15**, and **17** (Fig. 1).[7]

Analysis of heme binding to FVIII (Transfer onto protein level)

UV/Vis spectroscopic titrations of heme (18 µM) to full-length-FVIII (FL-FVIII; Octocog alpha; 900 nM) and B-domain-deleted-FVIII (BDD-FVIII; Simoctocog alfa; 900 nM) were performed to specify the heme-binding capacity of FVIII. This analysis revealed that both forms of FVIII, FL- and BDD-FVIII, are able to transiently bind up to seven heme molecules on their surface (Fig. 2).[7] Furthermore, heme binding occurred immediately upon mixture of FVIII with heme and stably over a time period of 60 min.[7]

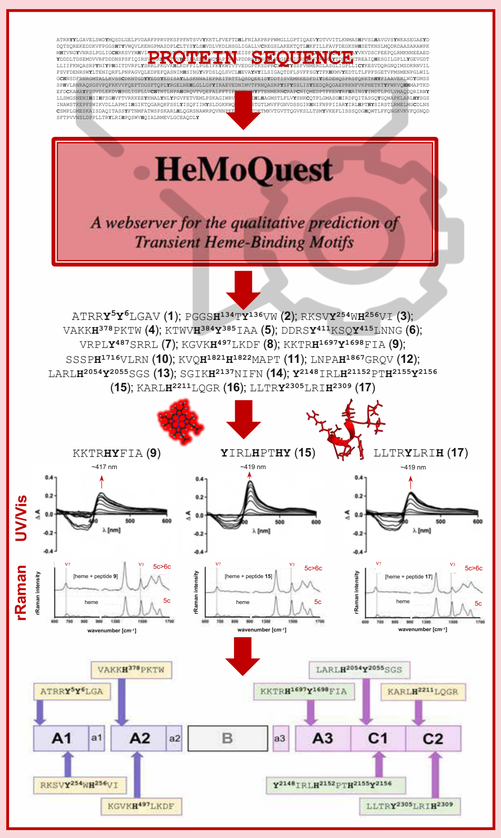

Figure 1. Prediction of HBMs by HeMoQuest and analysis of heme binding with FVIII-derived peptides as HBM models.[6,7]

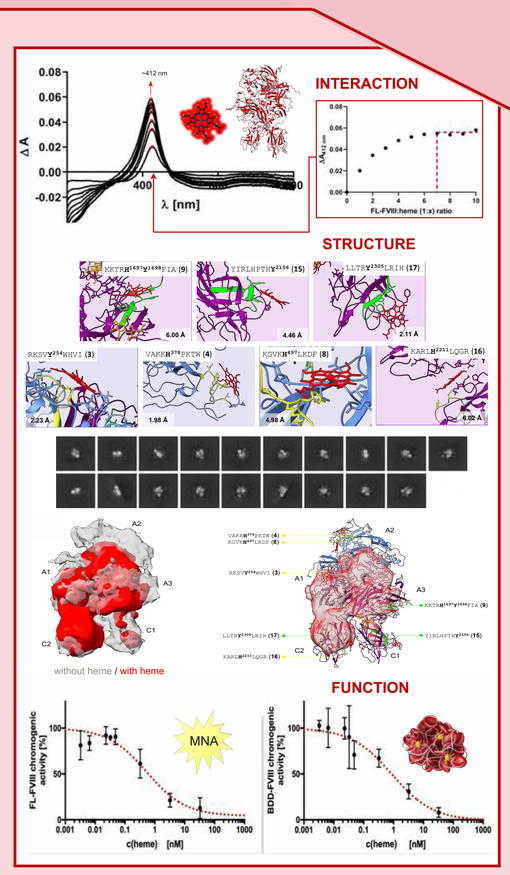

Figure 2. Heme binding affects the structural flexibility and functional activities of coagulation factor VIII.[7]

Cryo-EM and *in silico* characterization of the FVIII-heme complex

Heme was docked to FVIII (PDB: 2r7e) to each of the seven identified HBMs and remained there over a 50 ns molecular dynamics simulation, which confirmed stable binding of seven heme molecules to FVIII.[7] RMSF values suggested increased flexibility of distinct regions upon heme binding, which was further supported by single-particle cryo-EM-based studies (Fig. 2).[7] While FVIII (without heme) was able to form dimers, upon incubation with heme no dimers could be observed anymore. In contrast, only a core structure around the high affinity HBMs remained in structural map analysis, which (together with MALDI-TOF-MS and *in silico* analysis) suggests increased flexibility with a potential of functional consequences for the protein due to the importance of the affected regions for function-related essential protein-protein interactions.[7]

Impact of heme on the function of FVIII

In the presence of heme, the procoagulant cofactor activity of FVIII is inhibited in a chromogenic and a clotting assay with IC_{50} values of ~0.53 nM (FL-FVIII) and ~0.89 nM (BDD-FVIII) as well as ~3.44 nM (FL-FVIII) and ~1.54 nM (BDD-FVIII), respectively (Fig. 2).[7] These results show that heme tends to anticoagulant effects with respect to its interaction with FVIII.[7] More importantly, a potential immunomodulating effect of heme on FVIII will be tested in future.

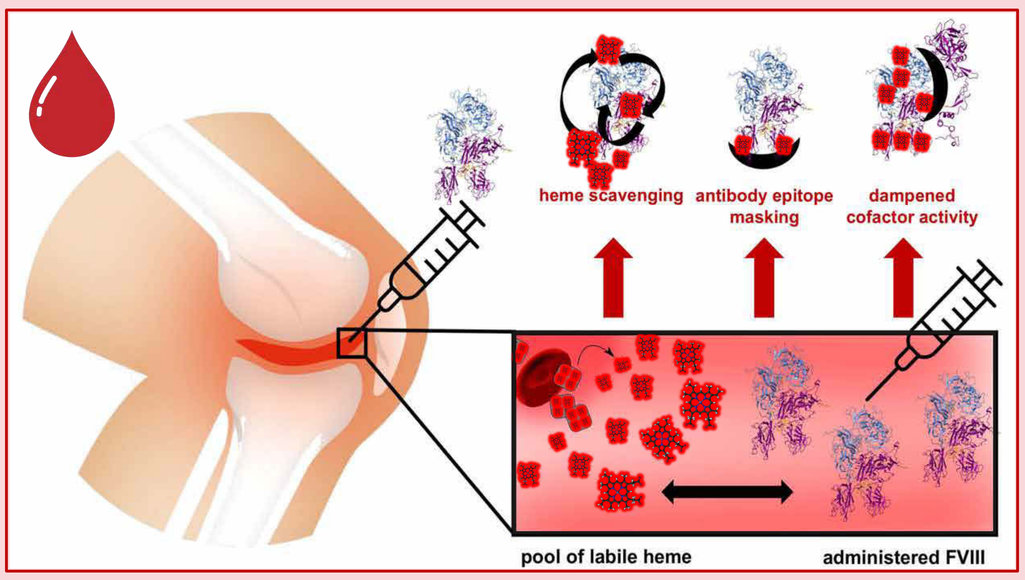

Figure 3. Overview of the potential consequences of the interaction between substituted (intraarticularly injected) FVIII and hemorrhage-derived heme.[7]

Conclusions

Heme binding to 19 FVIII-derived peptides was analyzed, revealing 7 HBMs. ● FVIII binds heme rapidly and stably. ● Heme binding induces structural flexibility in FVIII with functional consequences. ● Heme diminishes FVIII's procoagulant cofactor activity. ● Further studies are necessary to gain insights into the immunomodulating role of the FVIII-heme complex formation in FVIII substitution therapy of hemophilia A patients (Fig. 3). ● Model peptides will allow for the characterization of all blood coagulation proteins in future.

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