HISTIDINE-RICH GLYCOPROTEIN SIALYLATION AFFECTS ITS INHIBITION OF FXII AUTOACTIVATION

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Histidine-rich glycoprotein (HRG) is a multifunctional plasma protein modulating many biological processes, including coagulation and pathogen clearance. HRG is highly polymorphic, with five variations having minor allele frequencies (MAF) exceeding 10%, varying across populations. Additionally, HRG has 3 *N*-glycosylation sites (Asn63, Asn125, Asn344) and the Pro204Ser substitution introduces a potential *N*-glycosite at Asn202.
The autoactivation of Factor XII (FXII) plays a crucial role in initiating the contact pathway of coagulation. Recent studies have indicated that HRG might inhibit FXII autoactivation into FXIIa, which is the first step of the contact pathway of coagulation. Given the critical roles and connections of HRG and FXII in coagulation, we hypothesized that changes in sialylation and specific variations of HRG could affect FXII autoactivation.
We used LC-MS/MS-based (glyco)proteomics with HCD and stepped HCD fragmentation to characterize samples after IMAC and AEX chromatography purification. FXII autoactivation was measured using a S-2302 chromogenic assay, with OD at 405nm recorded continuously. MS results revealed that HRG was not the dominant protein in any fraction, as indicated by intensity analysis using Byonic software after purification. In each fraction, alpha-2-macroglobulin, albumin, hemopexin and other proteins were identified as the most abundant proteins. However, to the best of our knowledge, after consideration of the available literature, none of these proteins are expected to interfere with FXII autoactivation. We compared the relative abundance and profiling of HRG among all these fractions to select the fraction with the highest relative purity of HRG.
A kinetic FXII conversion bioassay showed that, the inhibition of autoactivation increased with higher HRG concentrations. Independent *t*-tests were performed to determine whether IC_{50} values from the samples of HRG with sialic acids differed significantly(p=0.0004).
We reveal that sialylation is an important component of HRG's role in inhibiting FXII autoactivation.