Pinpointing The Structural Dynamics of Plasminogen Activator Inhibitor -1 binding to Heparin using Hydrogen/Deuterium Exchange Mass Spectrometry

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Introduction

- PAI-1 is a key player to regulate the activation of fibrinolysis, with broad influence effects on inflammation, hemostasis, tissue remodeling, and wound healing[1].
- The binding of endogenous cofactor vitronectin to PAI-1 helps to extend PAI-1 half-life and delay its latency transition thus controlling the stability of the active form[1].
- Previous studies have suggested that low molecular weight heparin alters the levels of circulating PAI-1 and enhances endogenous fibrinolysis. However, the intrinsic dynamics of this binding are not completely understood[2].
- Our findings reveal that Low Molecular Weight Heparinn (LMWH) may contribute to the localization of PAI-1 at specific sites, hence involved in the regulation of plasminogen activation and its functional stability.
- Hydrogen-Deuterium Exchange (HDX) coupled to MS is widely used to study protein dynamics.
- Continuous time-resolved Electrospray ionization TRESI-HDX-MS technique is used to characterize protein structural transitions in relatively ordered regions of proteins[3].

Experimental Methods

- Recombinant human active PAI-1 stabilized by four mutations (14-1B), wild active PAI and Fondaparinux is a synthetic heparin pentasaccharide were obtained from Innovative Research Laboratories, and Amsbio respectively.
- Conformational dynamic studies using Continuous Flow Injection (CFI)TRESI-HDX-MS[3].
- Pumps were used flow rates 8 uL/min for the protein carrier buffer (100 mM ammonium Formate), 8 uL/min for the D_2O carrier buffer (100% H_2O), and 40 uL/min for the acid quench.
- Analytes were subjected to native mass spectrometry on a Waters G2S ion mobility mass spectrometer by direct infusion ESI.
- Data were collected on a Waters Synapt G2-Si and processed with Mass Spec Studio 1.0 and Mass Studio 2.0.

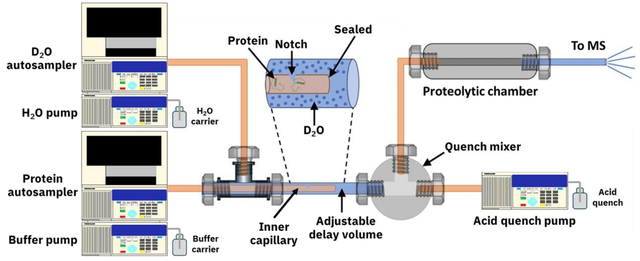
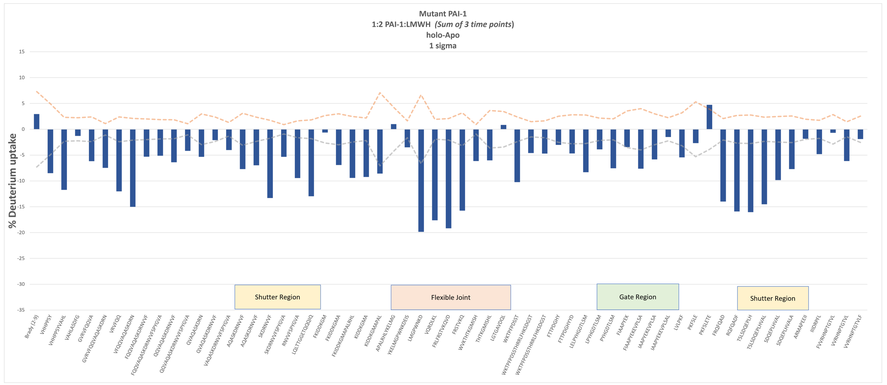


Figure 1. The continuous pullback device CFI-TRESI-HDX Coupled to Mass Spec utilized to perform automated data collection.

Results and Discussion

(A)



(B)

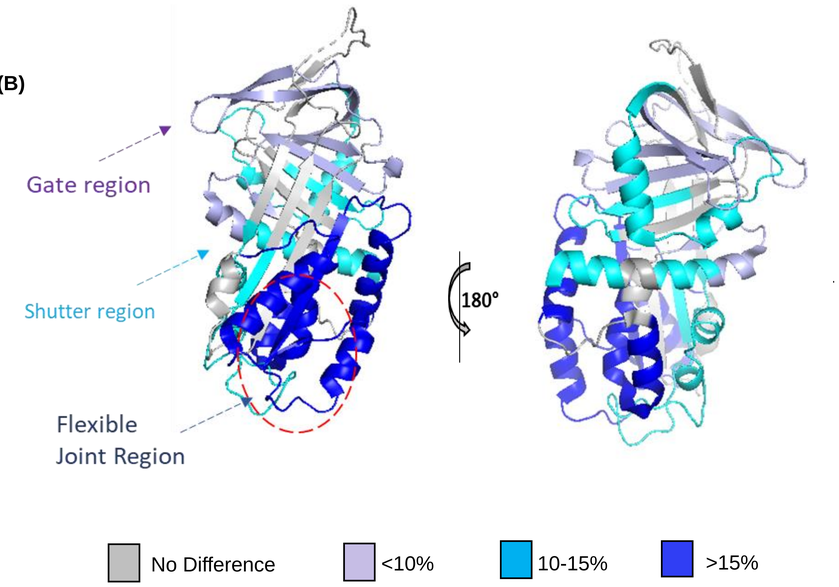


Figure 2. Maximal difference in Deuterium uptake with and without LMWH

(**A**) LMWH binding to PAI-1 induces a significant attenuation of deuterium uptake. Peptides covering the Flexible joint region has the strongest protection against isotopic exchange [peptides (99-105), (114-125)]. Some changes are also noticed in regions away from LMWH binding site, where modest decrease in D2o uptake take place [peptides (206-213), (239-249); gate area]. Lastly, Protection was also observed in Shutter area [peptide(307-319)]
(**B**) Differences in Deuterium uptake are mapped on 14-1B mutated PAI-1 structure, PDB 3Q02

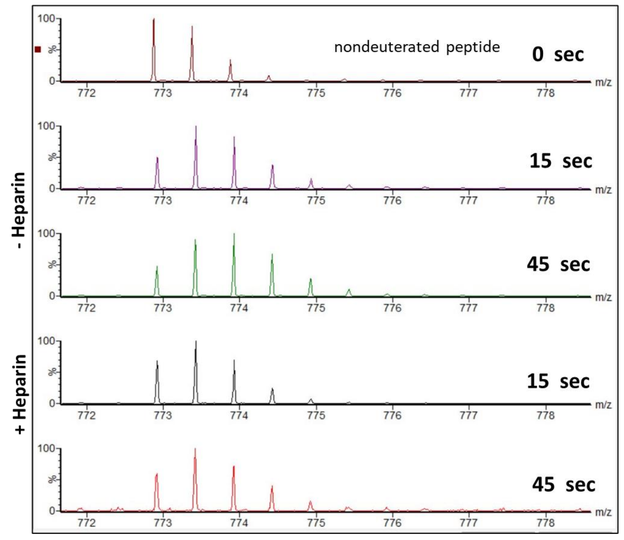


Figure 3. Example of a Deuterium –labeled PAI-1 peptide.
Deuterium Uptake Plot:- MS spectra of the peptide 46-59 (m/z 772.9) ion signal after HDX for 15 and 45 sec on PAI-1 in the presence and absence of LMWH. The spectrum of 0 sec represents nondeuterated peptide.

Conclusions and Future Work

- LMWH binding to active PAI-1 results in significant stabilization in the lower half PAI-1 molecule
- Upper few areas in PAI-1 Molecule are also impacted
- The binding sites of LMWH to 14-1B mutated PAI-1 is equal to the wild PAI-1 however more protection in observed in the mutated version.
- To further explore this study, we are going to investigate: longer time points conformational changes and kinetics of binding through transition between active and latent state.

References

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3) Anacleto, Joseph et al. “Apparatus for Automated Continuous Hydrogen Deuterium Exchange Mass Spectrometry Measurements from Milliseconds to Hours.” Analytical chemistry vol. 95,9 (2023): 4421-4428. doi:10.1021/acs.analchem.2c05003

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