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Mini Review

Mini-review: Heparin and Amyloid β2-Microglobulin

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Heparin is a major component of glycosaminoglycans (GAGs), which are components of tissues in the interstitial spaces of the body. Heparin is the most important anticoagulant used in clinical settings, especially for hemodialysis (HD).

Since Gejo's report in 1985, β_2-microglobulin (β_2-M) has been recognized as the precursor protein in dialysis-related amyloidosis (DRA), which is inevitably associated with long-term HD [1]. In general, this amyloidosis develops in the presence of two essential background conditions, i.e., extremely high concentrations of precursor proteins and amyloidogenic conformation of these precursor proteins. Connors [2] first demonstrated the amyloidogenic potential of β_2-M in 1985. Serum β_2-M levels in end-stage renal failure were known to be elevated because the kidney is the main organ related to the metabolism of β_2-M. In addition, we later proved that the conversion of the native β_2-M conformation to the amyloidogenic β_2-M conformation was triggered by unfolding of the C-terminal portion from Ile-92 to Met-99 [3, 4] (**Figure 1**).

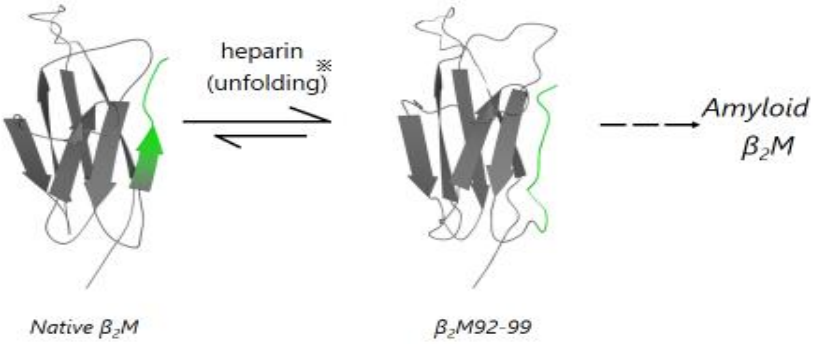

Figure 1

Figure 1: C-terminal unfolding : destructuring by loss of intramolecular H-bond involved in C-terminal 92-99

However, how native β_2-M is converted to an amyloidogenic conformer with the unfolded C-terminus in vivo, i.e., in clinical settings such as HD, and how β_2-M concentrations in the interstitial space reach as high as millimolar values despite micromolar serum levels of β_2-M in patients with HD [5] have not been clarified. In this mini-review, we would like to demonstrate that heparin may be a key molecule for the answers to both of these questions.

Study demonstrated a clear difference between HMH and LMH in interactions with β_2-M, as follows [7] .

Heparin is a mucopolysaccharide with different molecular weights (MWs) when prepared from animal organs, such as the intestine or the lung, and has been used widely as an anticoagulant. In the early 1990s, low-molecular-weight heparin (LMH), less than 6 kD, was introduced in HD to reduce the high risk of bleeding that had been associated with conventional heparin, which had heterogeneous MWs of more than 10 kD, or with high-molecular-weight heparin (HMH) [6]. Our previous

Binding of Heparin with β2-M

HMH binds in a dose-dependent manner with ΔN6β2-M, a well-known highly amyloidogenic variant of β2-M [8]; binds somewhat with D76N β2-M, a natural amyloid variant of β2-M; and demonstrates weak binding with native β2-M (**Figure 2**).

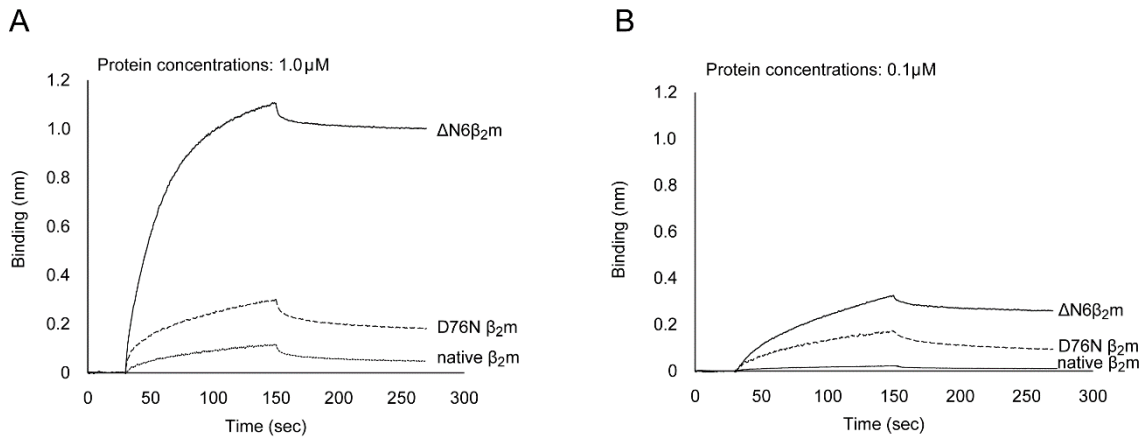

Figure 2: Analysis of the interaction of β2m variants with high molecular weight heparin.

Biotinylated H.M.H. (0.5 U/mL) was immobilized onto streptavidin biosensor. Protein concentrations were 1.0 μM (A) and 0.1 μM (B), respectively. ΔN6β2m (line, ——), D76N β2m (dashed line, ----) and native β2m (dotted line,).

The kD values for each β2-M species were as follows: 2.07×10^{-8}M, and 1.72×10^{-7}M, 3.71×10^{-6}M respectively. The kD value of ΔN6β2-M was low, consistent with that of its specific aptamer [7].

LMH, however, did not bind with ΔN6β2-M or native β2-M (**Figure 3**).

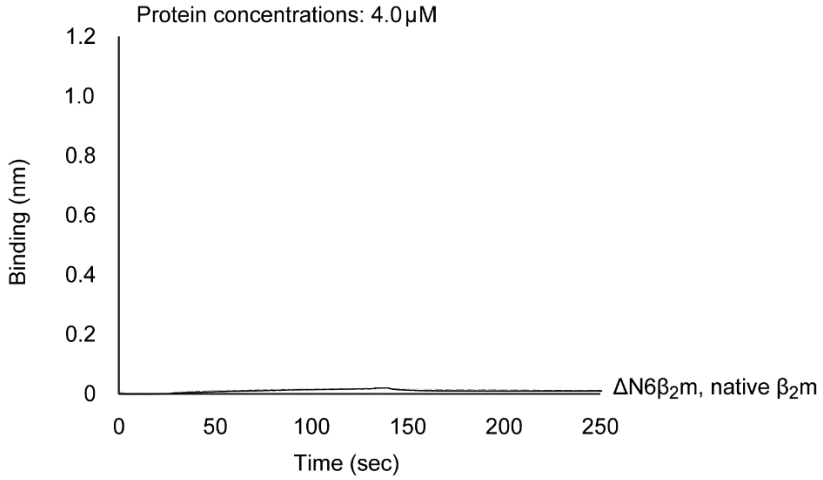

Figure 3: Analysis of the interaction of β2m variants with low molecular weight heparin.

Biotinylated L.M.H. (5.0 U/mL) was immobilized onto streptavidin biosensor. ΔN6β2m (line, ——) and native β2m (dotted line,) were used as the binding partner. Protein concentration was 4.0 μM. The real time binding curves were used to compute equilibrium dissociation constant by globally fitting the rate equation for 1:1 kinetics to the data. Three-independent experiments were performed, respectively.

Because GAGs in the interstitial space have a rich heparin component, β2-M has been trapped in the interstitial space and accumulated in a time-dependent fashion in patients undergoing HD, and their levels of β2-M are expected to reach as high as millimolar values in 10 years or more.

Unfolding of the C-terminus of the β2-M molecule

An intermediate molecule with a partially unfolded structure of precursor proteins was identified as a key molecule in the amyloidogenic process [10]. With regard to β2-M, we confirmed that the unfolding at the C-terminus from Ile-92 to Met-99 was key process for the initiation of amyloidogenicity of this precursor protein (**Figures 1–3**). Our study of heparin demonstrated that 1 μM HMH could trigger the C-terminal unfolding of β2-M, which was consistent with the serum concentrations of β2-M in patients undergoing HD (**Figure 4**) [7].

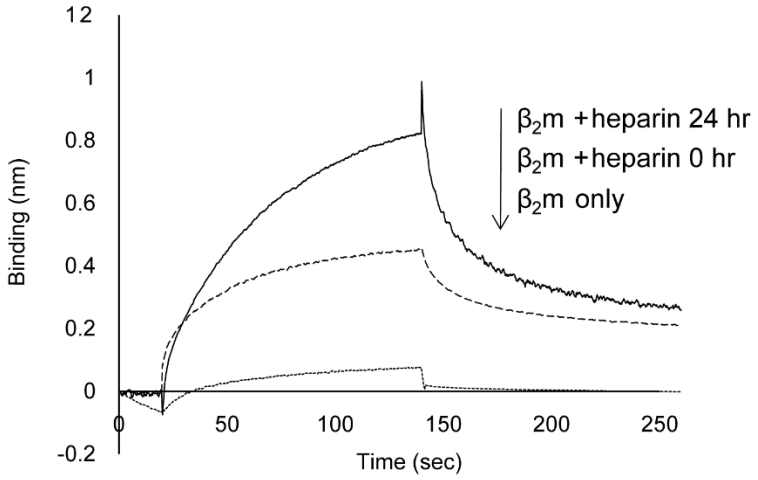

Figure 4: Analysis of the interaction of mAb92-99 with native β2m in the presence of H.M.H.

MAb92-99 (10 µg/mL) was immobilized onto protein a biosensor. After adding native β2m to the reaction drop holder in the presence or absence of H.M.H., the real-time binding was monitored. Native β2m incubated with H.M.H. (0.5 U/mL) for 24 h was also used as the binding partner.

In addition, given that β_2-M concentrations may be much higher in the interstitial space than are concentrations in serum, we may expect more unfolding at the N-terminus than the C-terminus, which is likely to undergo proteolysis at Lys-6 and generate a highly amyloidogenic variant, $\Delta N6\beta_2$-M [8], because the unfolding process is believed to proceed from the N-terminus of β_2-M [11].

GAGs Contain a Majority of the SO3– Moiety

Chemical solvents containing SO_3^- such as sodium dodecyl sulfate have induced conformational changes in proteins. For β_2-M, several studies indicated amyloidogenic conversion that depended on a majority of SO_3^- moieties being present in GAGs [12-15]. As is well-known, heparin is a highly negative mucopolysaccharide rich in the SO_3^- moiety. Although we had not studied the dose-dependent effect of heparin on the C-terminal unfolding of β_2-M, we believe that the SO_3^- majority contained in heparin may affect the results for HMH and LMH. The conformation of the β_2-Mmolecule is maintained by multiple intramolecular hydrogen bonds, which may be partially broken by multiple SO_3^- groups.

Conclusion

Collectively, our studies have indicated two actions of HMH with β_2-M: direct binding and induction of C-terminal unfolding. The former may result in an accumulation of β_2-M in the interstitial space and the latter may lead to β_2-M amyloidogenicity.

LMH showed no clear interactions, even with $\Delta N6\beta_2$-M, which indicates a superior clinical availability compared with HMH as an anticoagulant during long-term HD, in which DRA is a serious complication.

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