Nephrology and Renal Diseases

Review Article ISSN: 2399-908X

β2M shuttle hypothesis in the dialysis related amyloidosis (DRA)

Yoshihiro Motomiya[1]*, Yoshinori Uji[1] and Yukio Ando[2]

[1]Suiyukai Clinic, Kashihara, Nara, Japan

[2]Department of Amyloidosis Research, Nagasaki International University, Japan

β2-microglobulin(β2m) is the precursor protein of the dialysis related amyloidosis (DRA) in long term hemodialysis patients.

As well approved in current experimental studies over 20years or more, the intermediate molecules, i.e., the conformational variants of globular native protein, had been confirmed in the transitional process of the in vitro amyloidogenesis [1]. The presence of this molecule in hemodialysis (HD) setting had firstly reported in amyloid tissue from a femoral bone cyst in patient with the DRA by Bellotti's group [2]. Then, we had identified this intermediate β2microglobulin (I-β2M) using with capillary electrophoresis (C.E) in serum not only from HD patients but also the chronic kidney disease (CKD) patients and healthy persons, then, we proposed "β2M shuttle hypothesis " as amyloidogenic concept in clinical setting of HD [3,4]. This concept is based upon 5 evidences as follows:

1. A presence of I-β2M in serum,
2. Highly amyloidogenic variant, i.e. ⊿N6β2M as well as 92-99β2M found in the amyloid tissue, not in serum.
3. Aberrant I-β2M in post HD serum which is supposed to contain a part of interstitial fluid by rebound phenomenon,
4. Implication of glycosaminoglycan (GAGs) including heparin as promoting factors and
5. An alibi of amyloid β2M (Aβ2M) in serum.

We reviewed our concept herein again.

1) I-β2M; As confirmed with wildβ2M (Figure 1), β2M consist principally of 2 molecular components on C.E, i.e. one with major population of native molecule (N-β2M) and another with minor population of intermediate molecules (I-β2M), in the body fluid. Proportion of N-/I-β2M in serum vary roughly from 5 to 10, but no difference can be seen among patients with CKD, HD patients and healthy persons. However, HD provoke a drastic conversion from N-β2M to I-β2M and, consequently, post HD serum at 1 hour later ,which is supposed to contain at part the interstitial β2M, showed various C.E profile among individual cases but mostly showed profile with increased proportion of I-β2M accompanied by subpopulations of more cathodic β2M(I'-β2M) as shown in Figure 2 [4].

As for molecular structure, I-β2M consists of species with partially unfolded C-terminal, which can refold reversibly to N-β2M. Whereas, I'-β2M is supposed to consist of species with more, but not completely, unfolded C-terminal, which might be unlikely to refold to N-β2M.

2) The unfolding of the C-terminal; The conformational variant with the unfolded C-terminal from 92Ile to 99Met had been firstly demonstrated by Stoppini et al. and we proved to be present in amyloid tissue with monoclonal antibody in 2005 [5,6]. Few years later, we had also confirmed that the C-terminal of ⊿N6β2M was completely unfolded as same as 92/99β2M [7]. In addition, we had showed "smoking gun" evidence that heparin could provoke the C-terminal unfolding in native β2M at clinical doses in HD setting, demonstrating causative implication of interstitial GAG molecules in the C-terminal unfolding of β2M because heparin is one of main GAG molecules as matrix substance in the interstitial space [8].

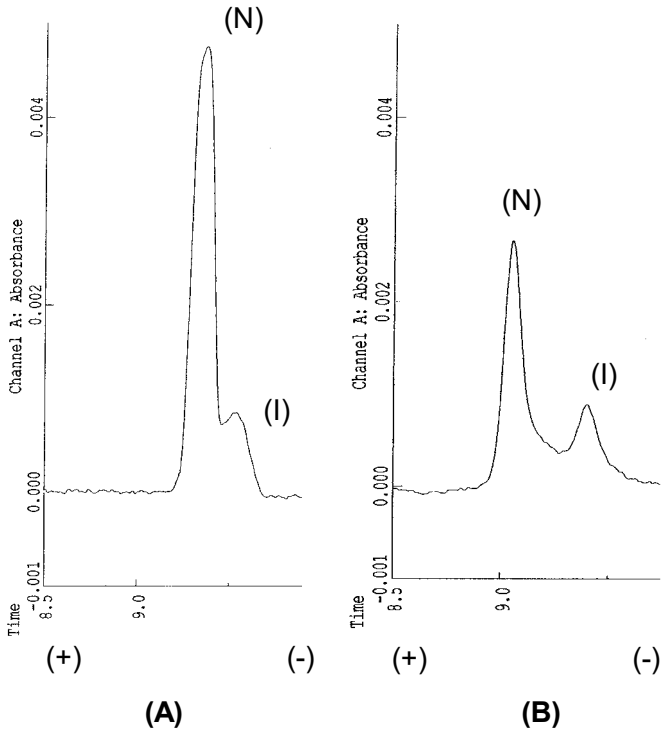

Figure 1. CE analysis of standard (purified human urine β2M)

(A) Standard β2M (Sigma); (B)treatment by 50% acetonitrile, peak 1: native β2M (N), peak 2: intermediate β2M (I-β2M).

Correspondence to: Y Motomiya, Suiyukai Clinic, 676-1 Kuzumoto-cho, Kashihara, Nara, 634-0007, Japan, E-mail: motomiya@silver.ocn.ne.jp

Received: November 29, 2019; **Accepted:** December 09, 2019; **Published:** December 12, 2019

3) ⊿N6β2M; ⊿N6β2M is a fragmental variant lacking 6 N-terminal amino acids which had been proved to be highly amyloidogenic and, therefore, be useful as model molecule for A-β2M [9]. ⊿N6β2M had firstly reported to be found in amyloid tissue from patients with the carpal tunnel syndrome and considered to be a degradation product by protease from N-β2M or I-β2M in the amyloid tissue [9,10]. The amyloidogenicity of ⊿N6β2M was directly proved by us using with the aptamer specific for ⊿N6β2M [11].

4) An alibi of A-β2M in serum; Amyloid proteins is an ultimately unfolded conformer of physiological native proteins including β2M which is a pivotal component of MHC-I and the precursor protein of this amyloidosis. Thus far, any kind of amyloid protein have not been reported in serum in any kind of amyloidosis. Similarly, as for β2M, we had also denied a presence of both A-β2M and ⊿N6β2M in serum from HD patients with LC/MS analysis as shown in Figure 3 [12]. However, charge state ions showed interesting differences between standard β2M (Sigma) and ⊿N6β2M. Our study imply that amyloid protein must be formed in extravascular space and cannot transfer crossing vascular wall, and more importantly, cannot be cleared via the kidney or even dialysis.

5) β2M shuttle concept in development of the DRA (Figure 4); β2M, both N-β2M and I-β2M, is considered to shuttle almost freely

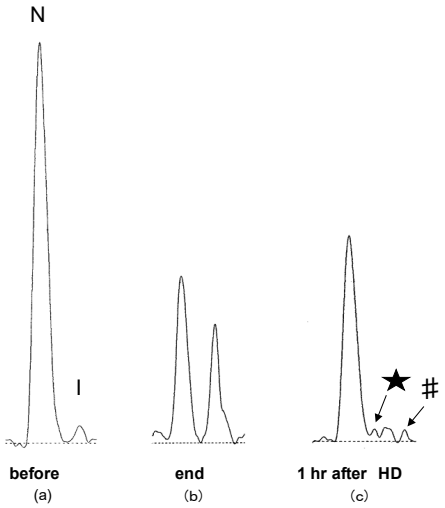

Figure 2. CE profiles.

at the start and the end of HD(b), and at 1hr after HD(c). (★) a peak on refolding, (#) a peak on more unfolding. [4]

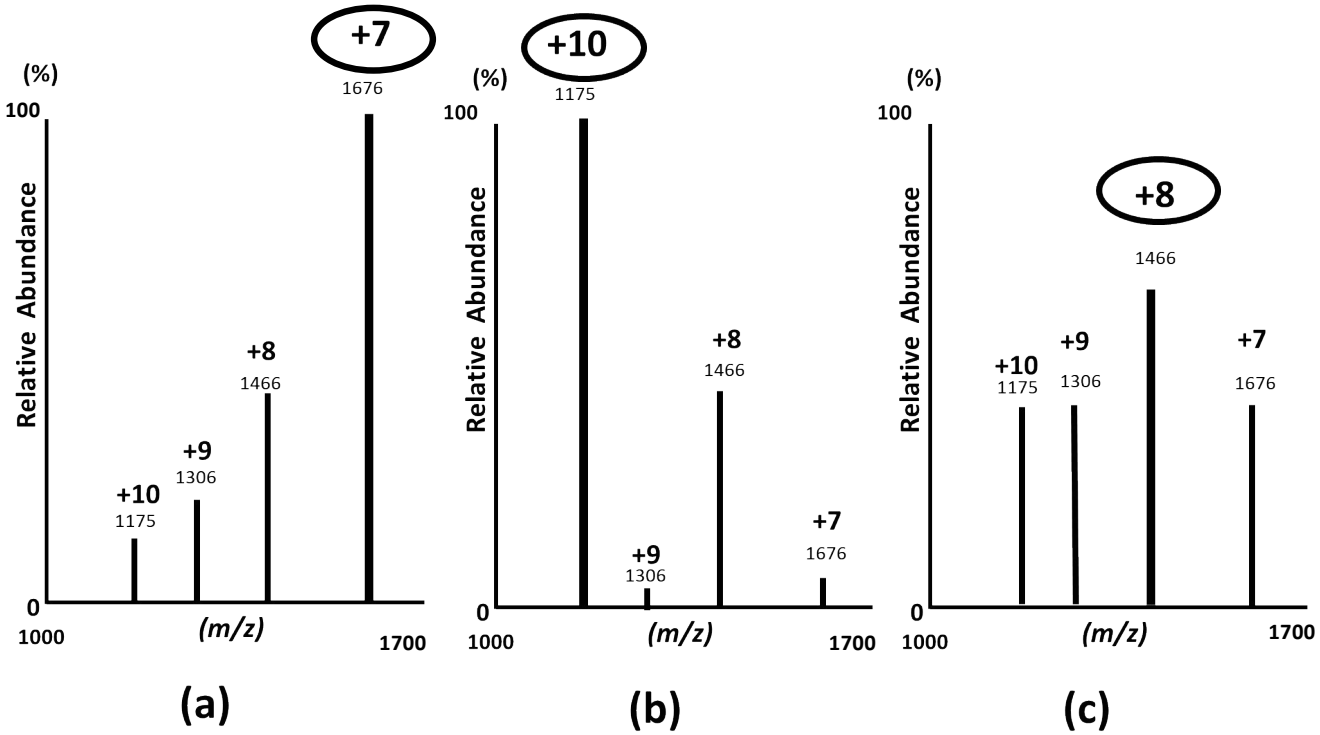

Figure 3. The *m/z* spectra of β2M, purified human urine β2M(Sigma) is centered at z=+7(a), ΔN6β2M is centered at z=+10 and uremic serum is centered at z=+8(c).

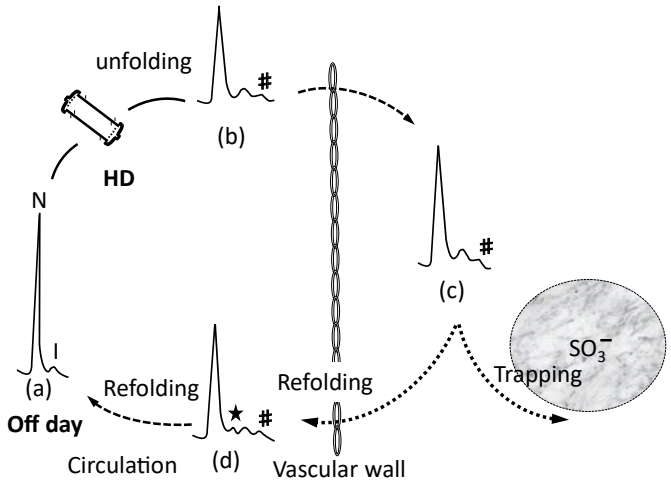

Figure 4. Illustrated dynamic shuttle of β2m. (4) (★) a peak on refolding, (#) a peak on more unfolding.

crossing over the vascular wall and the C-terminal of I-β2M in serum might be partially, not completely, unfolded. Whereas, in the interstitial space, there co-exist 3 kinds of β2M species, i.e., N-β2M, I-β2M and β2M92-99 with completely unfolded C-terminal, which might hard to refold into N-β2M. First, HD procedure, by itself, give rise to a conversion from N-β2M to I-β2M, both of which, then, undergo more unfolding at the C-terminal in the extravascular space, i. e, space rich of GAG molecules with SO3 moiety. Next, some I-β2M species interact with GAG to convert to β2M92-99, which can give rise to polymer and some I-β2M return into the vascular space and refold again to N-β2M [4].

In conclusion, β2M in serum exist ubiquitously at dynamic equilibrium between N-β2M and I-β2M with overwhelming predominance of N-β2M over Iβ2M. We believe that the presence of I-β2M with the unfolded C-terminal is "sine qua non" in development of the DRA, because a C-terminal unfolding could be also confirmed in the natural A-β2M, i.e, D76Nβ2M [8,13]. HD treatment provoke inevitably a drastic shift from N-β2M to I-β2M inside vascular wall and more unfolding at the C-terminal simultaneously outside of vascular wall. In addition, along with years of HD, the C-terminal of I-β2M transferred into the interstitial space have been becoming more unfolded and resulted in accumulation of β2M92-99, which lead to straightly a development of the DRA in the matrix space.

Reference

1. Chiti F, Manglone P, Andreola A, Sofia Giorgetti, Massimo Stefani, et al. (2001) Detection of two patially structured species in the folding process of the amyloidogenic protein, β2-microglobulin. *J Mol Biol* 307: 379-391.
2. Bellotti V, Stoppini M, Mangione P, Sunde M, Robinson C, et al. (1998) Beta2-microglobulin can be refolded into a native state from ex vivo amyloid fibrils. *Eur J Biochem* 258: 61-67. [Crossref]
3. Uji Y, Motomiya Y, Ando Y (2009) A circulating β2-microglobulin intermediate in hemodialysis patients. *Nephron Clin Pract* 111: c173-c181. [Crossref]
4. Motomiya Y, Uji Y, Ando Y (2012) Apillary electrophoretic profile of β2-microglobulin intermediate associated with hemodialysis. *Ther Apher Dial* 16:350-354. [Crossref]
5. Stoppini M, Bellotti V, Mangione P, Merlini G, Ferri G (1997) Use of anti-(beta2 microglobulin) mAb to study formation of amyloid fibrils. *Eur J Biochem* 249: 21-26. [Crossref]
6. Motomiya□Y, Ando Y, Haraoka K, Sun X, Morita H, et al. (2005) Studies on unfolded β2-microgulobulin at C-terminal in dialysis-related amyloidosis. *Kidney Int* 67: 314-320. [Crossref]
7. Motomiya Y, Higashimoto Y, Uji Y (2015) C-terminal unfolding of an amyloidogenic βII-microglobulin fragnment: ⊿N6β2-microglobulin. *Amyloid* 22: 54-60.
8. Fukasawa K, Higashimoto Y, Motomiya Y, Uji Y3, Ando Y4 (2016) Influence of heparin molecular size on the induction of C- terminal unfolding in β2-microglobulin. *Mol Biol Res Commun* 5: 225-232. [Crossref]
9. Esposito G, Michelutti R, Verdone G, Viglino P, Hernández H, et al. (2000) Removal of the N-terminal hexapeptide from human beta2-microglobulin facilitates protein aggregation and fibril formation. *Protein Sci* 9: 831-845. [Crossref]
10. Linke RP, Hampl H, Lobek H, Eberhard Ritz, Jürgen Bommer, et al. (1989) Lysine-specific cleavage of β2-microglobulin in amyloid deposits associated with hemodialysis. *Kidney Int* 36: 675-681.
11. Fukazawa K, Higashimoto Y, Ando Y, Motomiya Y (2018) Selection of DNA aptamer that blocks the fibrillogenesis of a proteolytic amyloidogenic fragment of β2m. *Ther Apher Dial* 22: 61-66. [Crossref]
12. Yoneda T, Hori S, Yoshida K (2019) Analysis of serum β2 microglobulin from hemodialysis patients using liquid chromatography/mass spectrometry. *J Jpn Soc Dial Ther* 52: 451-455.
13. Valleix S, Gillmore JD, Bridoux F, Mangione PP, Dogan A, et al. (2012) Hereditary systemic amyloidosis due to Asp76Asn variant β2-microglobulin. *N Eng J Med* 366: 2276-2283. [Crossref]

Copyright: ©2019 Motomiya Y. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.