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Review Article

The Therapeutic Potential of DNA Aptamer for Dialysis-Related Amyloidosis

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Abstract

β_2m is a precursor protein of the dialysis-related amyloidosis (DRA) and β_2m lacking the N-terminal six residues of the mature protein (ΔN6β_2m) is a one of amyloidogenic variant of β_2m. In the previous study, we had showed an unfolding in the C-terminal of ΔN6β_2m using the specific monoclonal antibody for ΔN6β_2m. We had also reported firstly an aptamer specific for ΔN6β_2m. ΔN6β_2m-aptamers specifically and tightly bound to ΔN6β_2m and inhibited fibril formation. These results suggest the potential of ΔN6β_2m-aptamers as reagents for therapeutic tools to prevent amyloid deposits in dialysis patients.

Keywords: dialysis-related amyloidosis; β_2-microglobulin; aptamer

Introduction

Amyloidosis is a misfolding disease of protein [1]. Every protein function adequately by taking eventually normal three-dimensional structure via folding process consisting of several intermediate molecules with partially unfolded structure [2]. Occasionally, a misfolded molecule which cannot refold into normal conformer in physiological condition happen to emerge among these intermediate molecules. A misfolded molecule is likely to aggregate each other and form amyloid fibril. β_2m is a precursor protein in the DRA [3]. In 1997, Stoppinni and Bellotti had reported that the C-terminal region from 92Ile-99Met in amyloid β_2m was completely unfolded [4]. Based upon their study, we have developed a monoclonal antibody specific for that region,i.e, mAb92-99 and proposed " β_2m shuttle hypothesis" as an underlying mechanism of the DRA [5, 6]. Using this mAb92-99, we had proved the C-terminal unfolding in ΔN6β_2m which was amyloidogenic variant of β_2m and lacking N-terminal 6 amino acids [7]. In addition, we also confirmed the C-terminal unfolding in another type of amyloid β_2m variant, Asp76Asn, reported by Valleix et al. [8]. Thus, we considered that the C-terminal complete unfolding must be common feature in amyloid β_2m. So, next, we have firstly explored an oligonucleotide-based aptamer specific for ΔN6β_2m (ΔN6β_2m-aptamer) [9]. We described here again characteristics and specificity of our ΔN6β_2m-aptamer.

1. ΔN6β_2m and mAb92-99.

β_2m variant lacking N-terminal 6 amino acids, ΔN6β_2m, had firstly found in amyloid tissue reported by Gejyo et al. in 1984 [10] and its high amyloidogenicity have been eventually proved by Esposito et al. in 2000 [11]. We had also demonstrated its unique structure profile differing from normal counterpart [11, 12] (Figure. 1). Meanwhile, we have demonstrated that our monoclonal antibody specific for the C-terminal region from 92Ile to 99Met, mAb92-99, exclusively reacted with ΔN6β_2m [7] (Figure. 2).

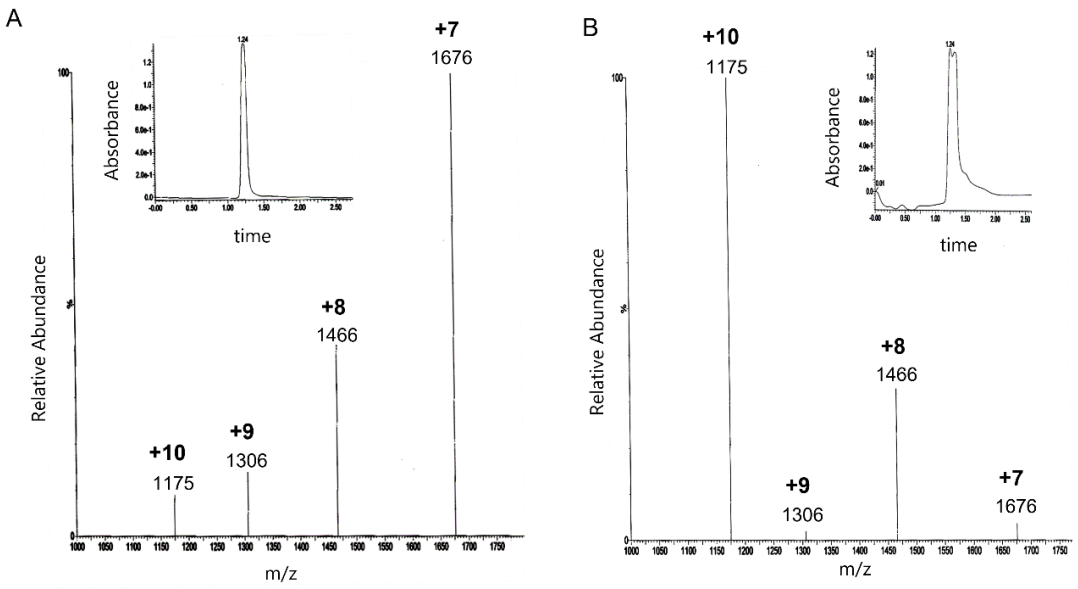

Figure 1: LC–MS analysis of β_2m. (A) standard β_2m (purified from human urine, Sigma) (B) ΔN6β_2m (in-house). Peak heights are reported as relative abundance, setting as 100 % the height of peak (A) and (B), respectively. (adapted from ref.12)

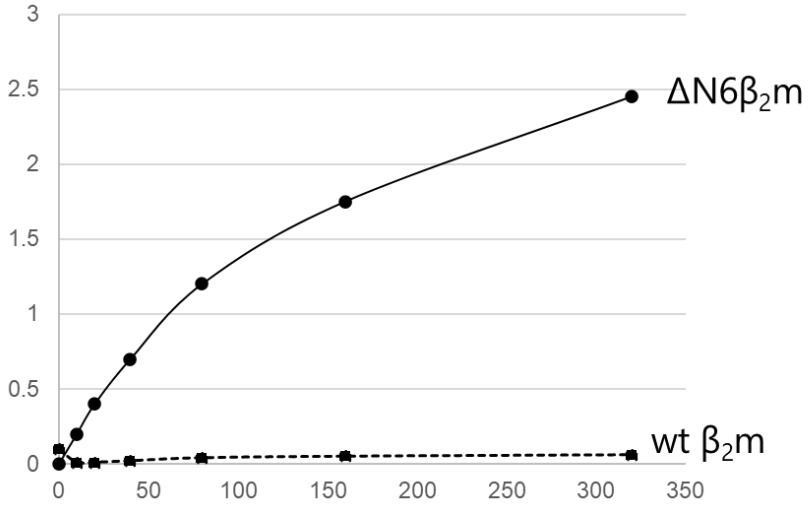

Figure 2: A sandwich ELISA of monoclonal antibody specific for the C-terminal region from 92Ile to 99Met, mAb92-99 (adapted from ref.7)

2. Aptamer vs ΔN6β_2m

Binding affinity of the ΔN6β_2m-aptamer with ΔN6β_2m was confirmed by biolayer interferometry analysis. As previously reported, the aptamer showed high affinity with ΔN6β_2m (Kd 23~55nM) and no reaction with normal β_2m on the other hand (Figure. 3)

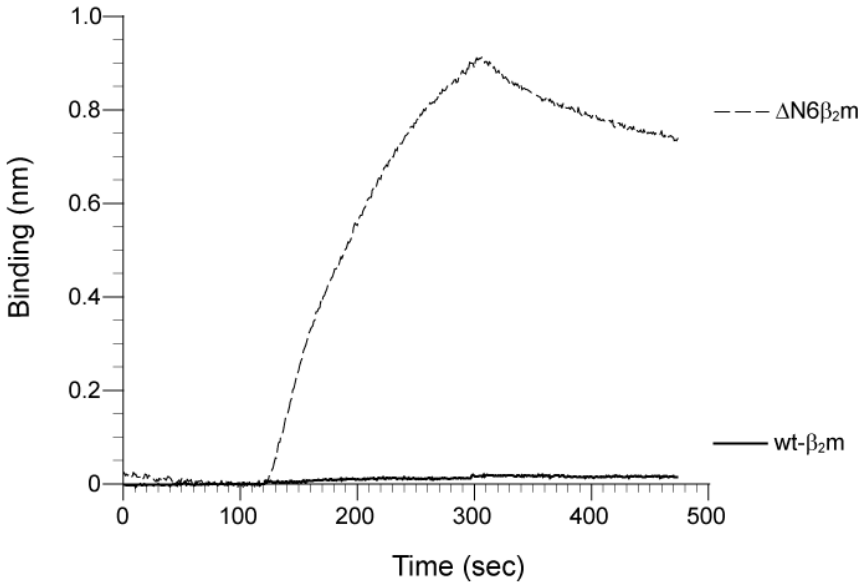

Figure 3: Binding affinity of ΔN6β2m-aptamer. Binding sensorgrams of ΔN6β2m-aptamer to immobilized ΔN6β2m and wt-β2m. The ΔN6β2m-aptamer were injected onto the sensor chip-immobilized ΔN6β2m (dashed lines) or wt-β2m (solid lines) at a concentration of 10 nM (adapted from ref.9).

3. Inhibition on amyloidogenesis

In addition, the ΔN6β2m-aptamer inhibited fibril formation in a dose-dependent manner, as assessed by Thioflavin T fluorescence assay (Figure. 4A). Fibrils formed from ΔN6β2m bind to Congo red, displaying changes in the absorbance spectrum of the dye characteristic of binding to amyloid fibrils, which was completely blocked by treatment with ΔN6β2m-aptamer (Figure. 4B).

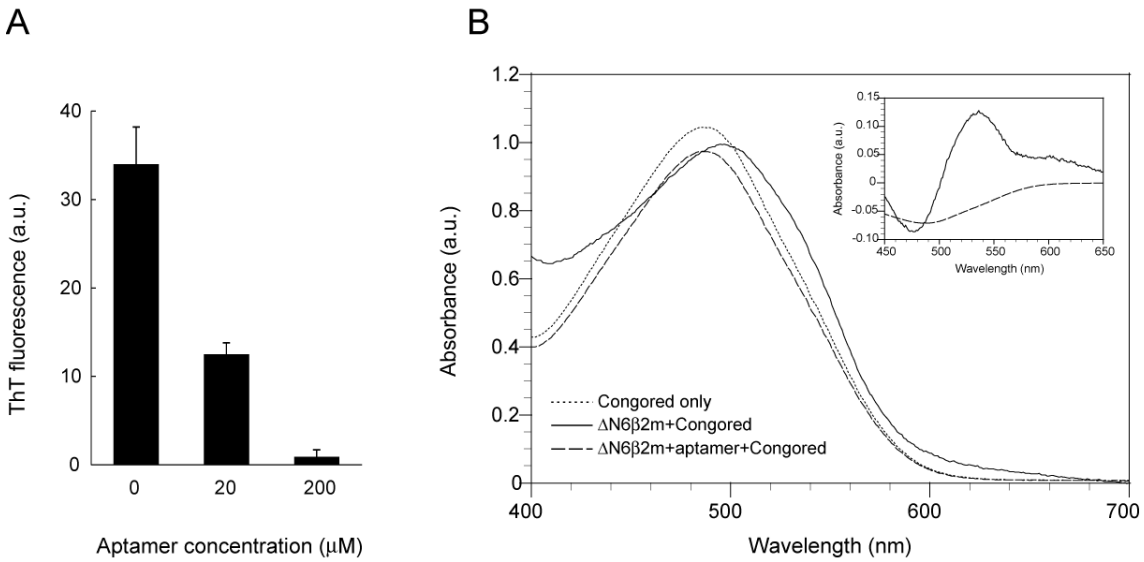

Figure 4: Effect of ΔN6β2m-aptamer on ΔN6β2m fibrillogenesis. (A) Fibril formation monitored by ThT fluorescence. ΔN6β2m (40 μM) was incubated in the absence or presence of 20 or 200 μM clone #8 aptamer for 2 weeks. (B) Absorbance spectrum of Congo red bound to ΔN6β2m fibrils formed in the presence or absence of ΔN6β2m-aptamer. Congo red alone (dotted line), ΔN6β2m without aptamer (solid line), and ΔN6β2m with aptamer (dashed line). The inset shows the difference spectrum ((Congo red + protein) - (Congo red alone)) for ΔN6β2m without aptamer (solid line) and ΔN6β2m with aptamer (dashed line). (adapted from ref.9)

4. Blocking effect on fibrillogenesis

In previous [9], we had showed macroscopic evidence of blocking on amyloid fibril formation by ΔN6β2m. We showed herein electroscopic proof of blocking effect of the ΔN6β2m-aptamer on fibrillogenesis as well. (Figure. 5)

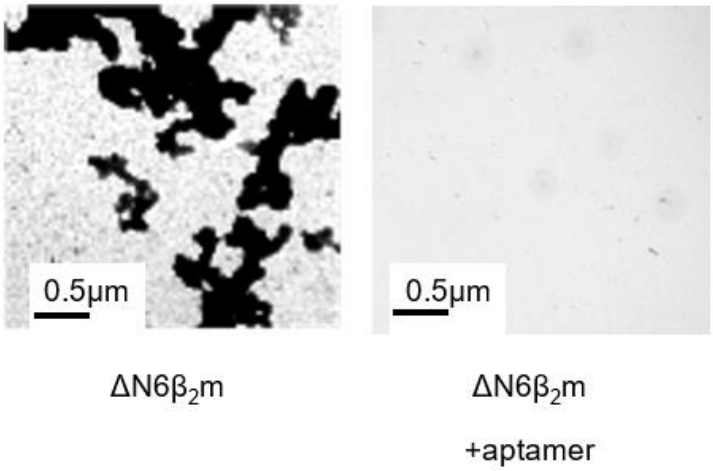

Figure 5: Fibrillogenesis ΔN6β2m followed by electron microscopy. ΔN6β2m (50 μM) was incubated for one week at 37ºC in the absence (right) or presence of ΔN6β2m-aptamer (50 μM) (left).

Current studies with highly sophisticated technology revealed undoubtedly that ΔN6β2m was a model molecule of amyloid β2m [10,11]. Our monoclonal antibody, mAb92-99, also reacted with another type of amyloid β2m, Asp76Asn variant β2-microglobin [8], as well as ΔN6β2m. Accordingly, we reasoned that a C-terminal complete unfolding was common feature with amyloid β2m. In addition, we considered that the C-terminal unfolding brings about irreversible conformational change on β2m molecule.

Nucleic acid aptamers are short single-stranded oligonucleotides that can bind to various kinds of target proteins like antibodies, which are selected by systemic evolution of ligands by exponential enrichment [13, 14]. Compared with neutralizing antibodies, aptamers are more easily prepared and could more efficiently penetrate various tissues with less immunogenicity and more thermal stability [13, 14]. Its advantages over antibodies for blocking target proteins make nucleic acid aptamers a very attractive tool for in vivo-therapeutic application. Indeed, pegaptanib (Macugen), an RNA-aptamer raised against vascular endothelial growth factor165, has already been approved by the U.S. Food and Drug Administration for the treatment of the wet type of age-related macular degeneration, while several types of aptamers directed against coagulation systems have undergone clinical trials [14].

Recently, we reported that DNA-aptamer directed against advanced glycation end products (AGEs) inhibited the binding of AGEs to its receptor (RAGE) and attenuated renal injury in obese type 2 diabetic mice [15, 16]. In addition, we have also found that the DNA aptamer raised against RAGE significantly blocks the binding AGEs, senescent macroprotein derivatives formed at an accelerated rate under diabetes, to RAGE and resultantly attenuates development and progression of experimental diabetic nephropathy, melanoma growth and metastasis, and renal and muscle injuries in animal models of chronic kidney disease [17–20]. These findings suggest that aptamers may be a therapeutic tool in the prevention of AGE–RAGE-related disorders.

As previously reported, our aptamer functioned analogous to mAb92-99, suggesting a possible inhibiting action on fibril formation by amyloid β2m [9]. Actually, as shown in Fig 5, ΔN6β2m-aptamer also showed definite inhibition on fibrillogenesis with electromicroscopic study as well as previous macroscopic study. Although a variety of aptamer have been developed since systematic evolution of ligands by exponential enrichment (SELEX) methodology had been established, their clinical application remains to be challenging. However, we believe an aptamer for amyloid β2m might be most possible therapeutic option for the DRA.

Conclusion

The role of ΔN6β2m in DRA is not currently understood. Although ΔN6β2m is present in the amyloid deposits found in patients with DRA, it remains unknown whether the N-terminal truncation of β2m occurs pre- or post-fibril formation. ΔN6β2m-aptamer may be useful as an analytical probe to derive greater clarity in understanding the early stages of β2m and ΔN6β2m co-assembly into amyloid.

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