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Utilization of a multiple antigenic peptide as a calibration standard in the BAN50 single antibody sandwich ELISA for Aβ oligomers

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ABSTRACT

Soluble amyloid-β (Aβ) oligomers are thought to be a cause of neurodegeneration and memory loss in Alzheimer disease (AD). We recently reported a newly developed enzyme linked immunosorbent assay (ELISA) for high molecular weight (HMW) Aβ oligomers in which the same Aβ monoclonal antibody, BAN50, was used for both capture and detection in a single antibody sandwich ELISA (SAS-ELISA) system. Our previous data suggest that this assay will be useful for the early diagnosis of AD, but its practical application to large-scale or longitudinal studies has been limited because of lack of a reliable calibration standard. In order to develop such a standard, we have now constructed a novel peptide using the multiple antigenic peptide (MAP) technique, where multiple epitopes of BAN50 were linked, *via* a spacer, to a branching lysine core. We show that the standard curve constructed from a 16-mer MAP covered the physiological range of signals obtained in the BAN50 SAS-ELISA from samples of human CSF, serum, and plasma. Furthermore, this 16-mer MAP is available in large quantities and is stable against freeze–thawing. We estimate that the signal per 1 pM of this standard corresponds to 1.54–5.0 pM of HMW Aβ oligomers. This MAP approach could also be used to provide an effective calibration standard for other SAS-ELISAs.

1. Introduction

Alzheimer disease (AD) is the most common cause of dementia and is a rapidly growing social and medical problem throughout the world. The two major pathological hallmarks in the brains of patients with AD are the senile plaques and neurofibrillary tangles [1]. The main protein component of the senile plaques is amyloid-β (Aβ) and that of neurofibrillary tangles is hyperphosphorylated tau protein [2,3]. Aβ1-42 is more prone to aggregation than Aβ1-40 and undergoes accelerated formation of Aβ oligomers, larger intermediate assemblies such as protofibrils, and, finally, insoluble amyloid fibrils [4]. The "amyloid cascade hypothesis" originally regarded these insoluble amyloid fibrils as the primary molecular culprit responsible for AD [5]. However, growing evidence supports the hypothesis that soluble Aβ oligomers (also described as ADDLs and/or protofibrils) are more toxic than the larger Aβ fibrils [6].

Aβ oligomers are heterogeneous assemblies vastly ranging in size. There is still no consensus on which type of oligomer is the most toxic pathogen in AD [7]. For instance, nanomolar concentrations of small, diffusible, synthetic Aβ oligomers (mainly 17–27 kDa) cause neuronal cell death in hippocampal slice cultures [8]. Cell-derived Aβ oligomers (mainly Aβ dimers) impair hippocampal long-term potentiation (LTP) in rat brains [9], and soluble Aβ oligomers (mainly Aβ dimers) from the brains of patients with AD inhibit LTP in normal mouse hippocampus and disrupt memory in normal rats [10]. The 56 kDa Aβ 12-mer (Aβ*56) purified from APP transgenic mice disrupts memory in young rats [11]. Among the Aβ oligomers tested, from monomers to tetramers, the synthetic Aβ tetramer was the most toxic species in cell culture studies [12].

Regardless of the size of toxic Aβ oligomers, many researchers anticipate that the selective measurement of Aβ oligomers in body fluids will have a better diagnostic value than that of non-selective detection of Aβ [13]. We have reported a novel enzyme linked immunosorbent assay (ELISA) system for the detection of Aβ oligomers in which the same anti-Aβ monoclonal antibody, BAN50, was used for both capture and detection of the oligomers, in a single antibody sandwich ELISA (SAS-ELISA) system. This BAN50 SAS-ELISA cannot detect Aβ monomers because the capture antibody occupies the only antibody-binding site available, but it can detect Aβ oligomers because they have multiple binding sites [14].

In a pilot study, our BAN50 SAS-ELISA specifically detected high molecular weight (HMW) Aβ oligomers of 10- to 20-mer in size, and gave signals in cerebrospinal fluid (CSF) samples from patients with AD or mild cognitive impairment that were significantly higher than those from age-matched controls, as well as having a

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negative correlation with Mini-Mental State Examination (MMSE) scores [15]. These facts suggest our SAS-ELISA can identify a useful molecular diagnostic marker for AD, and also a potential surrogate marker for disease severity. However, this ELISA system has a problem with its calibration standard. Conventionally, Aβ1-42 incubated at 4 °C overnight was used to provide a mixture of Aβ aggregates, including HMW oligomers, as the standard to adjust plate-to-plate signal variability. However, this type of Aβ 1-42 standard has a strong tendency to aggregate further during storage, handling, and the assay procedure itself, and also adheres to tubes and microtitre plates, giving problems with stability and uniformity. Consequently, it is not suitable for large-scale or longitudinal studies in which large numbers of samples are examined on different occasions in different experimental settings.

In the present study, to develop a reliable calibration standard for our SAS-ELISA, we have focused on a multiple antigenic peptide (MAP) approach. These MAPs consist of a synthetic peptide, containing the required epitope, which is linked (in this case *via* a short spacer arm) to a branching lysine core, to produce an 8 or 16-mer MAP [16]. These MAP systems were originally designed to produce multiple target antigens for efficient production of antibodies, and over the past two decades the techniques required for their synthesis have been well established. However, theoretically, these MAPs should be detected by an appropriate SAS-ELISA, because they have multiple copies of the required epitope and so could mimic protein oligomers.

The purpose of this study is to validate the reliability of MAP multimers as a calibration standard for the BAN50 SAS-ELISA.

2. Materials and methods

2.1. Sample collection

This study complied with the Declaration of Helsinki and was approved by the University Ethics Committee (Kyoto Prefectural University of Medicine, Kyoto, Japan). All subjects provided written informed consent to participate in this study. We collected CSF, serum, and plasma samples from nine patients with either clinically diagnosed AD ($n = 2$, sample number 1 and 2) or non-neurodegenerative disease ($n = 7$, sample number 3–9). Fresh samples were obtained from the enrolled subjects and then immediately stored at −80 °C until used for immunoassays.

2.2. MAP peptide

The linear peptide CGGGGSGDAEFRHDSGY (which we refer to here as the 'epitope-monomer') was synthesized by Peptide Institute (Osaka, Japan). The last 10 amino acids of this sequence, DAEFRHDSGY, correspond to Aβ1-10 and include the epitope for BAN50. The N-terminal part of the peptide, CGGGSG, is designed as a 'linker sequence' that should maintain the flexibility and accessibility of the BAN50 epitope to its antibody in aqueous solution. An 8-branched MAP (MAP 8-mer) and a 16-branched MAP (MAP 16-mer) were produced by Cambridge Peptides (Birmingham, UK) and Peptide Institute, respectively, by attaching the epitope-monomer to the required branching lysine core arrangement. To achieve this, amine groups on the lysine core were maleimidized and then coupled to the sulphydryl group on the N-terminal cysteine of the eptitope monomer (Fig 1A).

2.3. Preparation of Aβ oligomer mixtures from recombinant Aβ1-42

Aβ oligomer mixtures were prepared from recombinant Aβ1-42 (rPeptide, Athens, GA, USA). To avoid differences in aggregation state within the same batch of Aβ1-42 after storage, the peptide was first of all deseeded with trifluoroacetic acid (TFA) and hexafluoro-2-propanol (HFIP) according to the method described previously [17]. The deseeded Aβ1-42 was dissolved in dimethyl sulfoxide to yield a 1 mM concentration and further diluted with phosphate buffered saline without Ca^{2+} and Mg^{2+} (PBS(−): Sigma Aldrich, MO, USA) to 10 μM. Then, we incubated the solution at 4 °C for 24 h in order to generate Aβ oligomers. Although this solution may also contain some monomer, protofibrils or fibrils, we refer it as the 'parent Aβ oligomer mixture'.

2.4. Size-exclusion chromatography (SEC)

SEC was performed using an ÄKTApurifier System (GE Healthcare, MD, USA) with a column of Superdex 200 10/300GL (GE Healthcare). Molecular weight calibration was conducted in advance, using a high molecular weight gel filtration calibration kit (GE Healthcare). The monomeric and multimeric forms of Aβ1-42 contained in the parent Aβ oligomer mixture (500 μl, 10 μM) were separated with an elution buffer of PBS(−) at a flow rate of 0.5 ml/min, with absorbance being monitored at 280 nm. The eluted materials were collected as 1 ml fractions at 4 °C. To estimate the relative Aβ concentration in each fraction, their UV absorbance areas (arbitrary units) were calculated from the chromatogram, using Unicorn software (GE Healthcare). To avoid any further aggregation, the fractioned samples were subjected immediately after collection to the BAN50 SAS-ELISA for Aβ oligomers after five times dilution with chilled buffer (heat-inactivated phosphate buffer; 20 mM, pH 7.2 containing 10% BlockAce (Snow Brand, Tokyo, Japan), 0.2% BSA, 0.005% merthiolate Na, 400 mM NaCl, 0.076% CHAPS and 2 mM EDTA). The latter is the same composition as the sample dilution buffer used in the BAN50 SAS-ELISA. The Aβ concentration of each fraction was estimated from the UV absorption data. To determine the concentration of Aβ corresponding to each arbitrary unit of UV absorption area, deseeded Aβ1-42 solutions of different known concentrations were fractionated by the same SEC protocol as described above, and the areas under the peaks were determined. The correlation between the latter areas and the amount of Aβ loaded onto the column was strong enough to derive a formula to convert the chromatogram area to Aβ concentration (data not shown).

2.5. Measurement of Aβ oligomers with the BAN50 SAS-ELISA

The BAN50 SAS-ELISA was used in duplicate (unless otherwise stated) to measure the signal for Aβ oligomers in the eluted SEC fractions, the samples of biological fluids (CSF, plasma, serum) and in the MAP standards. The buffers and assay procedures were similar to those described previously [15]. To detect very small signals from the human body fluids, we used an auto-injector and a highly sensitive chemiluminescence substrate (Thermo Fisher Scientific, IL, USA), with luminescence detection on a multi-function microtitre plate-reader (Synergy 2, Biotek, VT, USA).

2.6. Statistics

Linear and non-linear regression was performed with GraphPad Prism 4.0 software (GraphPad, San Diego, CA, USA). Coefficient of determination (R^2) in the regression was also calculated using the same software.

3. Results

Fig. 1B shows the chemiluminescence signals obtained from the MAP 16-mer, the MAP 8-mer, and the epitope-monomer, in the BAN50 SAS-ELISA. It can be seen that the signals from the MAP

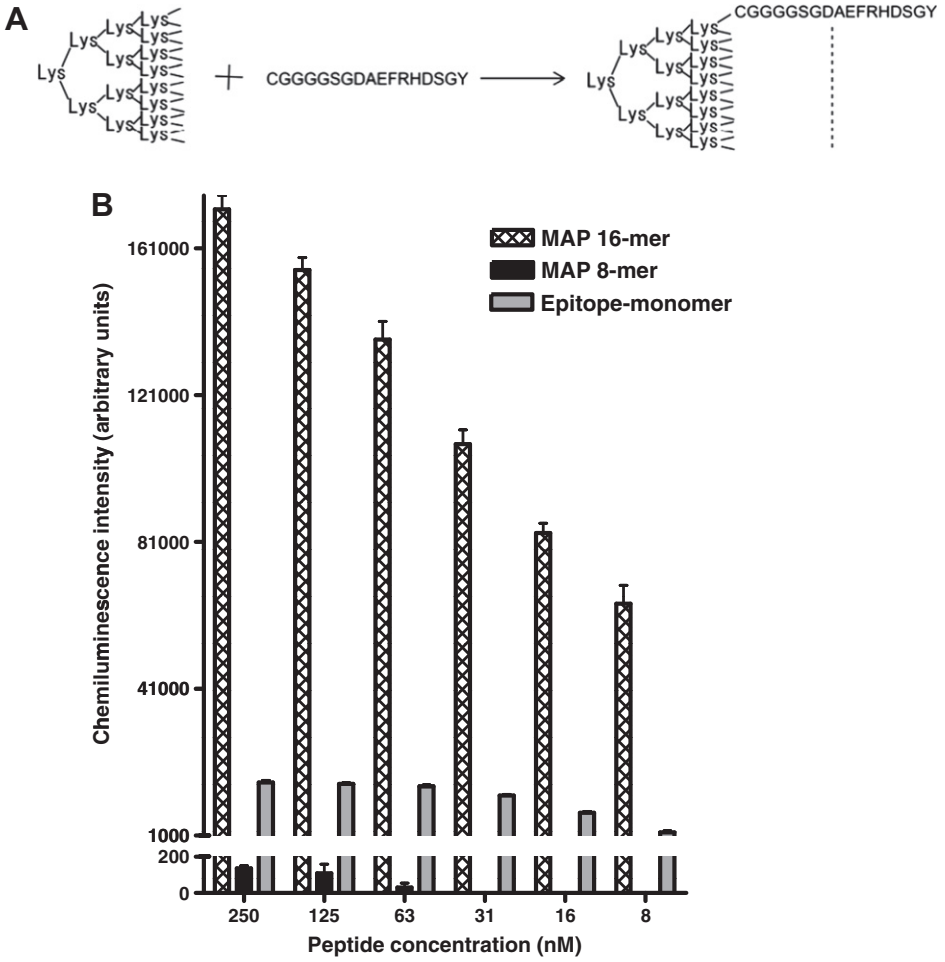

Fig. 1. (A) Schematic diagram illustrating linkage of the epitope-monomer, *via* its N-terminal cysteine, to the lysine core of the MAP, using a maleimide linker. (B) Chemiluminescence signals obtained by BAN50 SAS-ELISA from the MAP peptides and the epitope-monomer. Serially diluted samples of the MAP 16-mer, the MAP 8-mer, and the epitope-monomer were assayed. The *Y*-axis of the bar graph is divided into two segments (0–200 and 1000–180,000 chemiluminescence units) due to the extremely small signals obtained from the MAP 8-mer. Error bars indicate standard deviations.

16-mer were considerably higher than those obtained from either the MAP 8-mer or the epitope-monomer. The data clearly indicate that this SAS-ELISA system is much more efficient at recognizing the multiple epitope sequences in the MAP 16-mer than in the MAP 8-mer. The epitope-monomer gave an unexpectedly high signal that nearly reached 10% of that obtained with the MAP 16-mer. However, the concentration-dependence of the signal obtained from the MAP 16-mer was better than that obtained from the epitope-monomer, due to signal saturation seen at higher concentrations of the latter.

Fig 2A shows a standard curve obtained from the MAP 16-mer. It can be seen that the BAN50 SAS-ELISA successfully detected the MAP 16-mer, with an excellent goodness of fit (R^2 = 0.9943). Based on an increase in signal intensity of 3.3 times the standard deviation of the signal from blank wells, the detection limit of this ELISA was estimated to be 0.19 pM of the MAP 16-mer. To validate the clinical usefulness of the MAP 16-mer as a calibration standard, we ran it alongside CSF, serum and plasma samples from patients with AD and non-neurodegenerative disease controls (Fig 2B). The standard curve remained substantially linear over the low concentration range required for analysis of these samples (R^2 = 0.9924). The signals from the clinical samples ranged from 0.27 to 1.12 pM in CSF, from lower than the detection limit to 4.97 pM in serum, and from 0.22 to 0.67 pM in plasma (note that these signal levels are calculated based on the amount of MAP 16-mer required to give the same signal, unless otherwise stated). The intra-assay variability was less than 5% (the coefficient of variation (CV) = 4.3% at 312 pM, 3.0% at 156 pM, and 4.9% at 78 pM (n = 10)) and the inter-assay concordance against EC buffer containing 78 pM of MAP 16-mer was 6.1% (n = 5).

To examine stability of the MAP 16-mer, signal decrements against two cycles of freeze–thawing were measured at various concentration levels and compared with those of the parent Aβ oligomer mixture that was used as control in our previous report [15] (Fig 3). It can be seen that the signal variability of the MAP 16-mer against this treatment was much smaller (better) than that of the parent Aβ oligomer mixture at each concentration level, indicating that use of the MAP 16-mer standard can be expected to provide a more reliable calibration for the BAN50 SAS-ELISA.

To estimate how many units of the MAP 16-mer correspond to 1 pM of Aβ1-42 oligomers, we conducted SEC in combination with the SAS-ELISA (Fig 4). The parent Aβ oligomer mixture, prepared from Aβ1-42, was separated according to molecular size using SEC; the elution profile (OD 280 nm) revealed that a large part of the mixture consisted of fibrils and HMW oligomers. The lower part of Fig. 4 shows ELISA signals as represented by MAP 16-mer levels (pM) per unit of Aβ concentration (pM) in each fraction, as estimated from the UV absorption area data. In accord with our previous report [15], the BAN50 SAS-ELISA produced a significantly elevated signal from SEC fractions corresponding to HMW oligomers, and did not detect any signal in the fractions containing monomers, low molecular weight oligomers (LMW oligomers), or

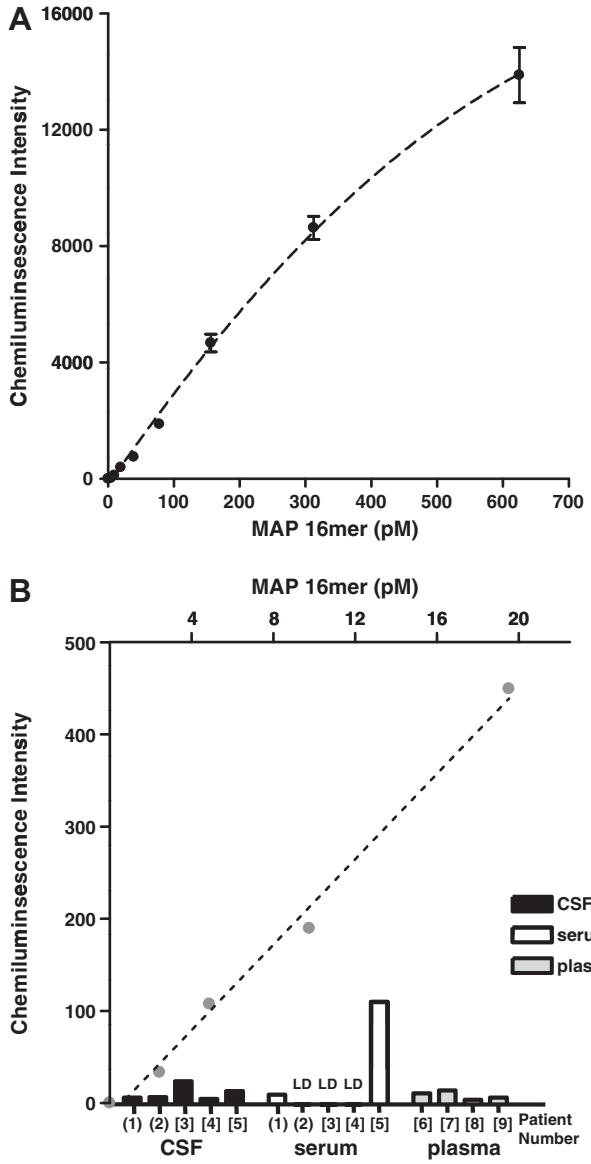

Fig. 2. (A) Standard curve for the MAP 16-mer in the BAN50 SAS-ELISA. Error bars indicate standard deviations. (B) Signal intensity from samples of CSF (white bars) in the BAN50 SAS-ELISA. Patient numbers in round parentheses represent AD cases and those in square brackets represent control cases. MAP 16-mer signals (gray circles) and its standard curve (dotted line) in the low concentration range are merged for reference.

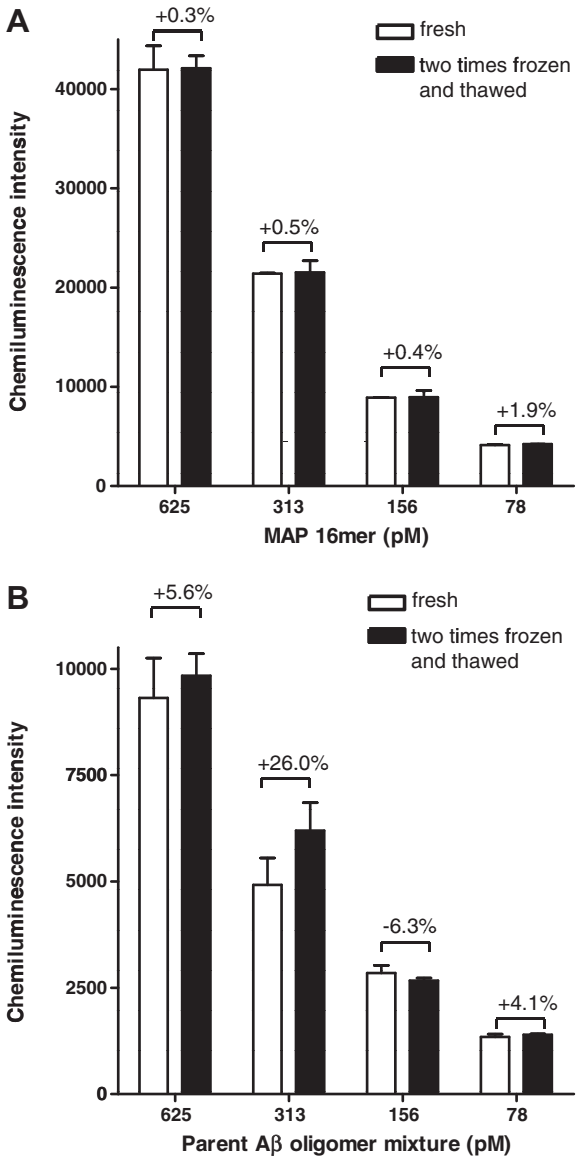

Fig. 3. The effect of two cycles of freezing and thawing on various concentrations of MAP. The *Y*-axis shows chemiluminescence (arbitrary units) and the *X*-axis shows the concentration of MAP 16-mer (pM). The white bars represent fresh material and the black bars represent the twice-cycled material. Signal decrement ratio (%) for two cycles of freeze–thawing is shown above each bar.

fibrils. The ratio of MAP 16-mer levels per unit of Aβ concentration varies depending on the sizes of the aggregates. However, in the fractions corresponding to HMW oligomers, the main target of this ELISA, all of these ratios fell within the range from 0.2 to 0.64. The highest ratio (0.64) was found in the fraction expected to contain Aβ 20-mer, in agreement with our previous result [15]. The results indicate that 1 pM of the MAP 16-mer can be estimated to give the same signal as 1.54–5 pM of HMW oligomers.

4. Discussion

Our first major findings are that the linear range of the standard curve calculated from the MAP 16-mer covers the readouts obtained from human biological samples (Fig 2B), and that the signals obtained using the MAP 16-mer are stable against at least two-cycles of freeze–thawing (Fig. 3). The MAP system is a well established method for producing high-titer anti-peptide antibodies [16]. A single peptide synthesis and purification producing 10–20 mg of MAP would be enough to prepare uniform calibration standards for over 300,000 microtitre plates. Moreover, the inter-assay and intra-assay coefficients of variation obtained using the MAP 16-mer were both lower than the generally acceptable level of 10%. These results suggest that standardization of the BAN50 SAS-ELISA using the MAP 16-mer would enable not only accurate measurements to be taken at a single facility, but also permit viable comparison of data across many different research institutions. Use of the MAP 16-mer standard should also allow longitudinal biomarker studies to be carried out, where there can be a need to compare data sets measured on different occasions.

Secondly, we show that the MAP 16-mer, but not the MAP 8-mer, gives a strong signal in the BAN50 SAS-ELISA (Fig. 1B). This observation is consistent with our previous finding that this ELISA specifically detects HMW Aβ oligomers in the size range 45–90 kDa

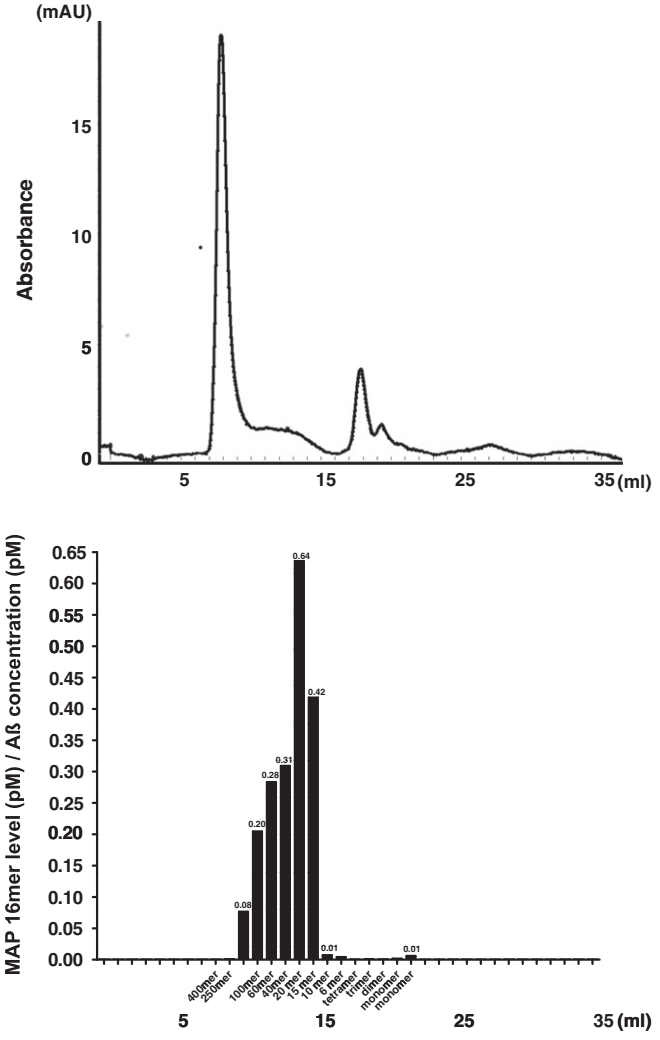

Fig. 4. SEC of parent Aβ oligomer mixtures made of Aβ1-42 (A) and the BAN50 SAS-ELISA for the eluted fractions (B). Black bars indicate ratios of ELISA signals represented by MAP 16-mer levels (pM) per Aβ concentration (pM) in each fraction, which were estimated based on UV absorption areas (280 nm). Ratios are shown above the bars. The scale of the *X*-axis representing elution volume in (B) is adjusted to that in (A).

(corresponding to 10- to 20-mer) as estimated by gel filtration studies, and does not detect low molecular weight (LMW) oligomers or the monomer [15]. This lack of signal with LMW oligomers has been observed with other SAS-ELISA systems, for instance the 4D8 SAS-ELISA for Aβ1-42 oligomers [14] and the C211 SAS-ELISA for α-synuclein oligomers [18], whereas some other SAS-ELISA systems are reported to detect LMW oligomers [19,20]. The explanation for this difference in ability to detect LMW oligomers amongst these various SAS-ELISA systems remains to be elucidated. However, hypothetically, we suppose that it is because the available epitopes for BAN50 are less exposed on the surface of LMW oligomers than HMW oligomers, due to large parts of them being embedded inside the 3-dimensional structure of LMW oligomers [15].

Thirdly, we found that moderate signals were generated from the epitope-monomer in the BAN50 SAS-ELISA (Fig. 1), even though this peptide does not have multiple epitopes. Although the average signal strength was no more than one tenth of that obtained from the MAP 16-mer, it did reach >100 times that obtained from the MAP 8-mer. Self-aggregation of the monomer peptide could provide an explanation for this finding. The sequence of the epitope-monomer is composed of a polyglycine–serine linker (CGGGGSG) attached directly to the N-terminal 10 amino acids of Aβ. The Aβ1-10 sequence, which includes the BAN50 epitope, has a relatively low tendency to self-aggregate compared to Aβ itself [21], but polyglycine does have inbuilt self-aggregating properties [22], and the N-terminal Cys confers the possibility of covalent cross-linking, *via* disulfide bond formation, to form stable multimeric structures. These facts imply that the linker peptide sequence could function as an aggregation 'core' and allow the epitope-monomer to generate a 'pseudo-positive' signal, by simulating a MAP multimer. Regardless of the explanation for this finding, it is clear that the BAN 50 SAS-ELISA does not detect Aβ monomers [15].

Finally, so that we can estimate the levels of Aβ oligomers from the standard curve constructed using the MAP 16-mer, we show data comparing the concentration of Aβ1-42 oligomers fractionated with SEC and the levels of the MAP 16-mer units in each fraction (Fig. 4). The major limitations of this analysis would be that the MAP 16-mer has a different structure from physiological Aβ oligomers and that these genuine Aβ oligomers consist not only of Aβ1-42 but also Aβ1-40 [23] and the three-dimensional structures as well as affinities of these two peptides for the BAN50 antibody are slightly different from each other [24]. Therefore, we should bear in mind that the accuracy of the conversion estimation cannot be guaranteed. However, these data do provide a rough indication of how to convert from raw data to real levels of Aβ oligomers.

For completeness, we should mention the accuracy of the estimate of total Aβ concentration obtained from the UV absorbance data. It may have been more appropriate to measure Aβ concentration in each SEC fraction using a commercial ELISA kit, and not by UV absorbance. However, unexpectedly, the Aβx-42 ELISA kit from WAKO (Osaka, Japan), which is generally used in this field, could not detect any signal from the fractions containing HMW oligomers while it could detect the LMW oligomers, or the monomer, as shown in the Supplementary Figure. However, the levels of Aβ1-42 were measured in the fractions containing LMW oligomers (from tetramer to monomer) using this kit, and they were approximately equal to those estimated based on the UV absorption areas (e.g. the average concentration from the tetramer fraction to the monomer fractions was 0.17 μM using the ELISA kit, which is very close to the value of 0.19 μM obtained from the UV absorbance data). Therefore, we can be reasonably confident that the Aβ1-42 levels estimated using the UV absorbance method are reliable.

In summary, we have demonstrated that the MAP system should provide an effective standard for the BAN50 SAS-ELISA. Increasing evidence suggests that soluble oligomers of aggregating proteins in neurodegenerative diseases are neurotoxic [25] and measurement of these oligomers should have better diagnostic value than non-selective measurement of the total concentration of each protein [13]. Techniques for the selective detection of oligomers can be roughly divided into two types. The first type is detection using conformational oligomer-selective antibodies or aggregate-specific ligands [23,26,27]. The other is the SAS-ELISA system that we have used [15,18,19,28]. The most important advantage of SAS-ELISA is that it can use existing monoclonal antibodies and is highly sensitive in detecting very small signals from oligomers in human body fluids if suitable antibodies are available. On the other hand, the disadvantage is the lack of reliable standards. A standard based on an antigen dimer, where a disulfide bond is formed between substituted cysteine residues within the antigen sequence, is one approach for a SAS-ELISA that can detect LMW oligomers [19]. However, SAS-ELISA systems do not always detect LMW oligomers [15,18,28]. For such ELISA systems, efficient, uniform and stable standards have not been developed. To our knowledge, this is the first report showing that the MAP

system is a useful approach for developing reliable standards for SAS-ELISA systems. An important advantage of the MAP calibration system is that it can support many varieties of epitope sequence, and we anticipate that this approach will provide effective calibration standards for other SAS-ELISAs aimed at detection of protein oligomers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.146.

References

[1] D.J. Selkoe, Alzheimer's disease: a central role for amyloid, J. Neuropathol. Exp. Neurol. 53 (1994) 438–447.
[2] D.J. Selkoe, Normal and abnormal biology of the β-amyloid precursor protein, Annu. Rev. Neurosci. 17 (1994) 489–517.
[3] M. Goedert, Tau protein and the neurofibrillary pathology of Alzheimer's disease, Trends Neurosci. 16 (1993) 460–465.
[4] J.T. Jarrett, E.P. Berger, P.T. Jr Lansbury, The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease, Biochemistry 32 (1993) 4693–4697.
[5] J. Hardy, D. Allsop, Amyloid deposition as the central event in the aetiology of Alzheimer's disease, Trends Pharmacol. Sci. 12 (1991) 383–388.
[6] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[7] S.L. Bernstein, N.F. Dupuis, N.D. Lazo, et al., Amyloid-β protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease, Nat. Chem. 1 (2009) 326–331.
[8] M.P. Lambert, A.K. Barlow, B.A. Chromy, et al., Diffusible, nonfibrillar ligands derived from Aβ 1-42 are potent central nervous system neurotoxins, Proc. Natl. Acad. Sci. USA 95 (1998) 6448–6453.
[9] D.M. Walsh, I. Klyubin, J.V. Fadeeva, et al., Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo, Nature 416 (2002) 535–539.
[10] G.M. Shankar, S. Li, T.H. Mehta, et al., Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory, Nat. Med. 14 (2008) 837–842.
[11] S. Lesne, M.T. Koh, L. Kotilinek, et al., A specific amyloid-β protein assembly in the brain impairs memory, Nature 440 (2006) 352–357.
[12] K. Ono, M.M. Condron, D.B. Teplow, Structure-neurotoxicity relationships of amyloid β-protein oligomers, Proc. Natl. Acad. Sci. USA 106 (2009) 14745–14750.
[13] S.A. Funke, Detection of soluble amyloid-β oligomers and insoluble high-molecular-weight particles in CSF: development of methods with potential for diagnosis and therapy monitoring of Alzheimer's disease, Int. J. Alzheimers Dis. 2011 (2011) 151645.
[14] H. LeVine III, Alzheimer's β-peptide oligomer formation at physiologic concentrations, Anal. Biochem. 335 (2004) 81–90.
[15] H. Fukumoto, T. Tokuda, T. Kasai, et al., High-molecular-weight β-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients, FASEB. J. 24 (2010) 2716–2726.
[16] D.N. Posnett, H. McGrath, J.P. Tam, A novel method for producing anti-peptide antibodies. Production of site-specific antibodies to the T cell antigen receptor β-chain, J. Biol. Chem. 263 (1988) 1719–1725.
[17] M. Taylor, S. Moore, J. Mayes, et al., Development of a proteolytically stable retro-inverso peptide inhibitor of β-amyloid oligomerization as a potential novel treatment for Alzheimer's disease, Biochemistry 49 (2010) 3261–3272.
[18] O.M. El-Agnaf, S.A. Salem, K.E. Paleologou, et al., Detection of oligomeric forms of α-synuclein protein in human plasma as a potential biomarker for Parkinson's disease, FASEB. J. 20 (2006) 419–425.
[19] W. Xia, T. Yang, G. Shankar, et al., A specific enzyme-linked immunosorbent assay for measuring β-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease, Arch. Neurol. 66 (2009) 190–199.
[20] A.C. Klaver, L.M. Patrias, J.M. Finke, et al., Specificity and sensitivity of the Aβ oligomer ELISA, J. Neurosci. Methods 195 (2011) 249–254.
[21] L.O. Tjernberg, J. Naslund, F. Lindqvist, et al., Arrest of β-amyloid fibril formation by a pentapeptide ligand, J. Biol. Chem. 271 (1996) 8545–8548.
[22] S. Ohnishi, H. Kamikubo, M. Onitsuka, et al., Conformational preference of polyglycine in solution to elongated structure, J. Am. Chem. Soc. 128 (2006) 16338–16344.
[23] C.M. Gao, A.Y. Yam, X. Wang, et al., Aβ40 oligomers identified as a potential biomarker for the diagnosis of Alzheimer's disease, PLoS One 5 (2010) e15725.
[24] B. Urbanc, L. Cruz, S. Yun, et al., In silico study of amyloid β-protein folding and oligomerization, Proc. Natl. Acad. Sci. USA 101 (2004) 17345–17350.
[25] C. Haass, D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide, Nat. Rev. Mol. Cell. Biol. 8 (2007) 101–112.
[26] D.G. Georganopoulou, L. Chang, J.M. Nam, et al., Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease, Proc. Natl. Acad. Sci. USA 102 (2005) 2273–2276.
[27] N. Schupf, M.X. Tang, H. Fukuyama, et al., Peripheral Aβ subspecies as risk biomarkers of Alzheimer's disease, Proc. Natl. Acad. Sci. USA 105 (2008) 14052–14057.
[28] T. Tokuda, M.M. Qureshi, M.T. Ardah, et al., Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease, Neurology 75 (2010) 1766–1772.