2Pos041

Development of thio-NAD cycling ELISA for detection of Aβ42 and 40

アミロイドβ42と40検出のためのチオNADサイクリングELISA法の開発

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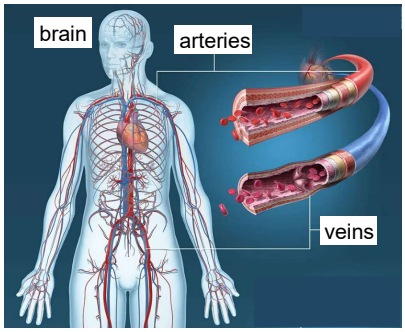
Abstract

Recently, blood biomarkers have attracted attention in the early diagnosis of Alzheimer's disease (AD). Representative biomarkers are amyloid β-protein 42 and 40. However, the amount of these proteins excreted from the brain into the blood is extremely small, so it is essential to develop a detection method for this purpose. In the present study, we report that it has become possible to measure Aβ42 and Aβ40 easily and with ultrahigh sensitivity using a thio-NAD cycling ELISA that combines a sandwich ELISA method and an enzyme cycling method.

Introduction

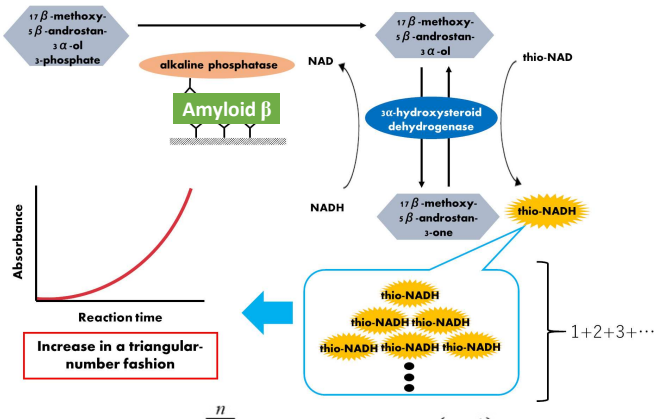
At present, mass spectrometry is used to measure extremely small amounts of Aβ42 and Aβ40. However, the measurement method is complicated and the equipment is expensive, so an easy-to-use and reasonable cost method is needed. This research proposes the development of a measurement technique for this purpose.



A tiny amount of Aβ42 and Aβ40 produced in the brain leaks into the blood of both AD patients and healthy individuals. Therefore, it is thought that diagnosis can be made by measuring the amount of this protein in the blood.

Methods

Proteins cannot be amplified. Our idea, thus, amplifies the detectable signal for a trace amount of proteins. We combined a sandwich ELISA with another method, i.e., a thio-NAD cycling method. The cycling enzyme used was 3α-hydroxysteroid dehydrogenase (3α-HSD). Thio-NAD is reduced to thio-NADH, which can be measured directly by an increase in the absorbance at 400 nm. Our method can detect alkaline phosphatase (ALP) at the order of 10^{-21} moles. The thio-NADH signal intensity is expressed as a triangle number.

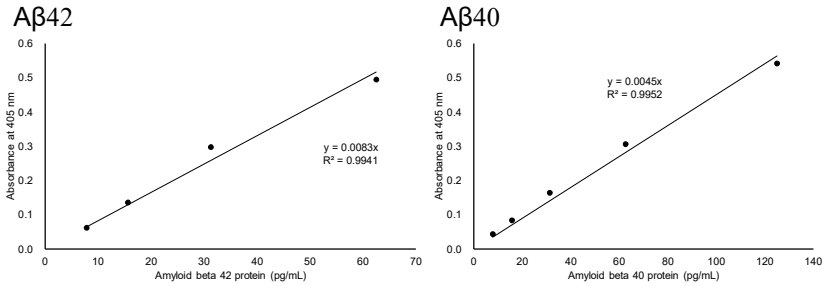


$$a \times b \times \sum_{k=1}^{n} k = a \times b \times \frac{n(n+1)}{2}$$

Here *a* is the turnover ratio of ALP per minute, *b* is the cycling ratio of 3α-HSD per minute, and *n* is measurement time in minutes.

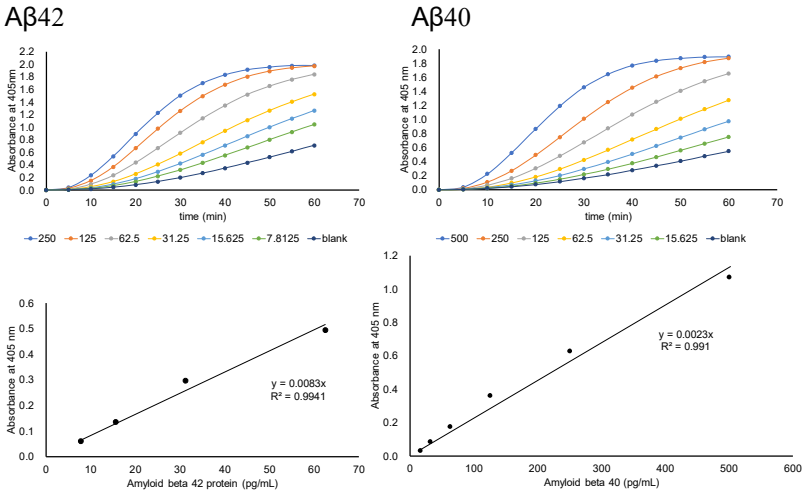
Results

Measurements of Aβ42 and Aβ40 in buffer



	Aβ42	Aβ40
LOD (pg/mL)	0.29	0.31
LOD (moles/assay)	6.5×10^{-18}	7.2×10^{-18}
LOQ (pg/mL)	0.98	1.04
LOQ (moles/assay)	2.1×10^{-17}	2.4×10^{-17}

Measurements of Aβ42 and Aβ40 in control serum



	Aβ42	Aβ40
LOD (pg/mL)	1.60	3.25
LOD (moles/assay)	3.5×10^{-17}	7.5×10^{-17}
LOQ (pg/mL)	5.35	10.8
LOQ (moles/assay)	1.1×10^{-16}	2.5×10^{-16}

Discussion

In the future, it is necessary to detect tactual AD patient samples using our method. Compared to the results of previous studies, the measuring sensitivity is expected to be sufficient to measure Aβ42 and Aβ40 in blood. Our measurement method only detects absorption at 400 nm, which is visible light, and is simple and inexpensive, requiring only a small measuring device and making it possible to perform measurements at the patient's bedside.

Messages from BioPhenoMA:

- For detailed data on previously published research, please contact us and we will explain it at a separate meeting.
- It is possible for us to perform trial measurements on samples that your company or research institution has (detailed discussions are required).